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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 13:19:56 ON 17 MAY 2004

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 13:20:22 ON 17 MAY 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s ubiquitin conjugating enzyme# or ubc##
FILE 'MEDLINE'
          9906 UBIQUITIN
          2174 CONJUGATING
        691967 ENZYME#
           910 UBIQUITIN CONJUGATING ENZYME#
                 (UBIQUITIN(W)CONJUGATING(W)ENZYME#)
           794 UBC##
L1         1233 UBIQUITIN CONJUGATING ENZYME# OR UBC##

FILE 'SCISEARCH'
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          2388 CONJUGATING
        430085 ENZYME#
           821 UBIQUITIN CONJUGATING ENZYME#
                 (UBIQUITIN(W)CONJUGATING(W)ENZYME#)
           956 UBC##
L2         1416 UBIQUITIN CONJUGATING ENZYME# OR UBC##

FILE 'LIFESCI'
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           891 "CONJUGATING"
        190694 ENZYME#
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                 ("UBIQUITIN"(W)"CONJUGATING"(W)ENZYME#)
           406 UBC##
L3          566 UBIQUITIN CONJUGATING ENZYME# OR UBC##

FILE 'BIOTECHDS'
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           264 CONJUGATING
        113700 ENZYME#
            55 UBIQUITIN CONJUGATING ENZYME#
                 (UBIQUITIN(W)CONJUGATING(W)ENZYME#)
            41 UBC##
L4           82 UBIQUITIN CONJUGATING ENZYME# OR UBC##

FILE 'BIOSIS'
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          2424 CONJUGATING
        740726 ENZYME#
           791 UBIQUITIN CONJUGATING ENZYME#
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           879 UBC##
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FILE 'EMBASE'
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          1902 "CONJUGATING"
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           694 UBIQUITIN CONJUGATING ENZYME#
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FILE 'HCAPLUS'
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          4347 CONJUGATING
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FILE 'NTIS'
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            65 CONJUGATING
         11898 ENZYME#
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            95 UBC##
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          1094 CONJUGATING
        206645 ENZYME#
           505 UBIQUITIN CONJUGATING ENZYME#
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           599 UBC##
L9         840 UBIQUITIN CONJUGATING ENZYME# OR UBC##

FILE 'BIOTECHNO'
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        353854 ENZYME#
           562 UBIQUITIN CONJUGATING ENZYME#
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FILE 'WPIDS'
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         72727 ENZYME#
            73 UBIQUITIN CONJUGATING ENZYME#
                 (UBIQUITIN(W)CONJUGATING(W)ENZYME#)
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TOTAL FOR ALL FILES
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FILE 'SCISEARCH'
L14        186 L2 (5A)GENE/Q

FILE 'LIFESCI'
L15        160 L3 (5A)GENE/Q
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FILE 'BIOTECHDS'
L16          22 L4  (5A)GENE/Q

FILE 'BIOSIS'
L17         258 L5  (5A)GENE/Q

FILE 'EMBASE'
L18         161 L6  (5A)GENE/Q

FILE 'HCAPLUS'
L19         458 L7  (5A)GENE/Q

FILE 'NTIS'
L20           0 L8  (5A)GENE/Q

FILE 'ESBIOBASE'
L21         139 L9  (5A)GENE/Q

FILE 'BIOTECHNO'
L22         163 L10 (5A)GENE/Q

FILE 'WPIDS'
L23          17 L11 (5A)GENE/Q

TOTAL FOR ALL FILES
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FILE 'SCISEARCH'
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L26         131 L2  (5A)HUMAN

FILE 'LIFESCI'
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L27          70 L3  (5A)HUMAN

FILE 'BIOTECHDS'
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L28          37 L4  (5A)HUMAN

FILE 'BIOSIS'
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L29         168 L5  (5A)HUMAN

FILE 'EMBASE'
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FILE 'NTIS'
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FILE 'ESBIOBASE'
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FILE 'BIOTECHNO'
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FILE 'WPIDS'
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L35         24 L11(5A)HUMAN

TOTAL FOR ALL FILES
L36       1127 L12(5A) HUMAN

=> s 124 and 136
FILE 'MEDLINE'
L37         36 L13 AND L25

FILE 'SCISEARCH'
L38         36 L14 AND L26

FILE 'LIFESCI'
L39         35 L15 AND L27

FILE 'BIOTECHDS'
L40         15 L16 AND L28

FILE 'BIOSIS'
L41         62 L17 AND L29

FILE 'EMBASE'
L42         34 L18 AND L30

FILE 'HCAPLUS'
L43        155 L19 AND L31

FILE 'NTIS'
L44          0 L20 AND L32

FILE 'ESBIOBASE'
L45         30 L21 AND L33

FILE 'BIOTECHNO'
L46         33 L22 AND L34

FILE 'WPIDS'
L47         10 L23 AND L35

TOTAL FOR ALL FILES
L48        446 L24 AND L36

=> s 148 not 2001-2004/py
FILE 'MEDLINE'
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L49         31 L37 NOT 2001-2004/PY

FILE 'SCISEARCH'
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L50         32 L38 NOT 2001-2004/PY

FILE 'LIFESCI'
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L51         28 L39 NOT 2001-2004/PY

FILE 'BIOTECHDS'
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L52         11 L40 NOT 2001-2004/PY
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FILE 'BIOSIS'
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L53         43 L41 NOT 2001-2004/PY

FILE 'EMBASE'
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L54         30 L42 NOT 2001-2004/PY

FILE 'HCAPLUS'
      3387196 2001-2004/PY
L55         85 L43 NOT 2001-2004/PY

FILE 'NTIS'
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L56          0 L44 NOT 2001-2004/PY

FILE 'ESBIOBASE'
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L57         26 L45 NOT 2001-2004/PY

FILE 'BIOTECHNO'
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L58         30 L46 NOT 2001-2004/PY

FILE 'WPIDS'
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L59          5 L47 NOT 2001-2004/PY

TOTAL FOR ALL FILES
L60        321 L48 NOT 2001-2004/PY

=> fil .becpat
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                     109.38     109.59

FILES 'BIOTECHDS, HCAPLUS, WPIDS' ENTERED AT 13:28:11 ON 17 MAY 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

3 FILES IN THE FILE LIST

=> s l48 and wo/pc and pry<=2000 and py>=2001 range=2003,
FILE 'BIOTECHDS'
         11351 WO/PC
          3766 PRY<=2000
                 (PRY<=2000)
         38913 PY>=2001
                 (PY>=2001)
L61          0 L40 AND WO/PC AND PRY<=2000 AND PY>=2001

FILE 'HCAPLUS'
         72554 WO/PC
         27803 PRY<=2000
       1390820 PY>=2001
L62          0 L43 AND WO/PC AND PRY<=2000 AND PY>=2001

FILE 'WPIDS'
        160862 WO/PC
        143856 PRY<=2000
                 (PRY<=2000)
       1234392 PY>=2001
                 (PY>=2001)
L63          0 L47 AND WO/PC AND PRY<=2000 AND PY>=2001
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TOTAL FOR ALL FILES
L64 0 L48 AND WO/PC AND PRY<=2000 AND PY>=2001

=> log y
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	7.55	117.14

STN INTERNATIONAL LOGOFF AT 13:29:23 ON 17 MAY 2004

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1006	ubiquitin adj conjugating adj enzyme$1 or ubc$2	US-PGPUB; USPAT	OR	OFF	2004/05/17 10:57
L3	730766	gene$1 or sequence$1	US-PGPUB; USPAT	OR	OFF	2004/05/17 10:57
L4	140	1 near5 3	US-PGPUB; USPAT	OR	OFF	2004/05/17 10:58
L5	317	1 same human	US-PGPUB; USPAT	OR	OFF	2004/05/17 10:58
(L6)	88	4 and 5	US-PGPUB; USPAT	OR	OFF	2004/05/17 10:58

priority to 10/30/00

PGPUB-DOCUMENT-NUMBER: 20040072170

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040072170 A1

TITLE: Novel target genes for diseases of the heart

PUBLICATION-DATE: April 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bunk, Daniela Beck nee	Iffeldorf		DE	
Reuner, Birgit	Munich		DE	
Beck, Joachim	Iffeldorf		DE	
Henkel, Thomas	Kassel		DE	

APPL-NO: 10/ 276775

DATE FILED: February 25, 2003

PCT-DATA:
APPL-NO: PCT/EP01/06165
DATE-FILED: May 30, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6

ABSTRACT:

The present invention relates to a variety of genes abnormally expressed in heart tissue as well as to fragments of such genes. Assessment of the expression level of these genes may be used for testing the predisposition of mammals and preferably humans for a heart disease or for an acute state of such a disease. Preferred diseases in accordance with the invention are congestive heart failure, dilative cardiomyopathy, hypertrophic cardiomyopathy and ischemic cardiomyopathy. The present invention further relates to methods of identifying compounds capable of normalizing the expression level of the aforementioned genes and of further genes affected by the abnormal expression. The identified compounds may be used for formulating compositions, preferably pharmaceutical compositions for preventing or treating diseases. They may also be used as lead compounds for the development of medicaments having an improved efficiency, a longer half-life, a decreased toxicity etc. and to be employed in the treatment of heart diseases. Included in the invention are also somatic gene therapy methods comprising the introduction of at least one functional copy of any of the above-mentioned genes into a suitable cell. Finally, the invention relates to non-human transgenic animals comprising at least one of the aforementioned genes in their germ line. The transgenic animals of the invention may be used for the development of medicaments for the treatment of heart diseases.

---------- KWIC ---------

Detail Description Paragraph - DETX (197):

[0393] The prey showed 100% identity with the human Ubc9 sequence the clone covered the all Ubc9 sequence. Ubc9 is thought to be involved in the ubiquitin-dependent protein degradation system (Wang et al. 1996). A single copy of the hUBC9 gene was found and localised to human chromosome 16p13.3. Interestingly the interaction of Daxx (see above) was already found with the Ubc9 protein (Ryu et al., 2000).

PGPUB-DOCUMENT-NUMBER: 20040053388

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040053388 A1

TITLE: Detection of protein conformation using a split ubiquitin reporter system

PUBLICATION-DATE: March 18, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Eckert, Jorg H.	Witten		DE	
Johnsson, Nils	Eggenstein-Leopoldshafen		DE	
Raquet, Xavier	Xhendelesse		BE	

APPL-NO: 10/ 250614

DATE FILED: July 2, 2003

PCT-DATA:
APPL-NO: PCT/US02/00325
DATE-FILED: Jan 3, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/194, 435/320.1 , 435/325 , 435/69.7 , 530/350 , 530/399

ABSTRACT:

The invention provides methods and reagents for monitoring protein structure by an intrapolypeptide split-ubiquitin assay.

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application 60/259827, filed on Jan. 4, 2001, the specifications of which is incorporated by reference herein.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (143):

[0194] The term "ubiquitin" as used herein refers to an abundant 76 amino acid residue polypeptide that is found in all eukaryotic cells. The ubiquitin polypeptide is characterized by a carboxy-terminal glycine residue that is activated by ATP to a high-energy thiol-ester intermediate in a reaction catalyzed by a ubiquitin-activating enzyme (E1). The activated ubiquitin is transferred to a substrate polypeptide via an isopeptide bond between the activated carboxy-terminus of ubiquitin and the epsilon-amino group of a lysine residue(s) in the protein substrate. This transfer requires the action of ubiquitin conjugating enzymes such as E2 and, in some instances, E3 activities. The ubiquitin modified substrate is thereby altered in biological function,

and, in some instances, becomes a substrate for components of the ubiquitin-dependent proteolytic machinery which includes both UBP enzymes as well as proteolytic proteins which are subunits of the proteasome. As used herein, the term "ubiquitin" includes within its scope all known as well as unidentified eukaryotic ubiquitin homologs of vertebrate or invertebrate origin which call be classified as equivalents of human ubiquitin. Examples of ubiquitin polypeptides as referred to herein include the human ubiquitin polypeptide which is encoded by the human ubiquitin encoding nucleic acid sequence (GenBank Accession Nunbers: U49869, X04803). Equivalent ubiquitin polypeptide encoding nucleotide sequences are understood to include those sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants as well as sequences which differ from the nucleotide sequence encoding the human ubiquitin coding sequence due to the degeneracy of the genetic code. Another example of a ubiquitin polypeptide as referred to herein is murine ubiquitin which is encoded by murine ubiquitin encoding nucleic acid sequence (GenBank Accession Number: X51730). It will be readily apparent to the person skilled in the art how to modify the methods and reagents provided by the present invention to the use of ubiquitin polypeptides other than human ubiquitin.

Brief Description of Drawings Paragraph - DRTX (147):

[0198] The term "ubiquitin conjugation machinery" as used herein refers to a group of proteins which function in the ATP-dependent activation and transfer of ubiquitin to substrate proteins. The term thus encompasses: E1 enzymes, which transform the carboxy-terminal glycine of ubiquitin into a high energy thiol intermediate by an ATP-dependent reaction; E2 enzymes (the UBC genes), which transform the E1-S.about.Ubiquitin activated conjugate into an E2-S.about.Ubiquitin intermediate which acts as a ubiquitin donor to a substrate, another ubiquitin moiety (in a poly-ubiquitination reaction), or an E3; and the E3 enzymes (or ubiquitin ligases) which facilitate the transfer of an activated ubiquitin molecule from an E2 to a substrate molecule or to another ubiquitin moiety as part of a polyubiquitin chain. The term "ubiquitin conjugation machinery", as used herein, is further meant to include all known members of these groups as well as those members which have yet to be discovered or characterized but which are sufficiently related by homology to known ubiquitin conjugation enzymes so as to allow an individual skilled in the art to readily identify it as a member of this group. The term as used herein is meant to include novel ubiquitin activating enzymes which have yet to be discovered as well as those which function in the activation and conjugation of ubiquitin-like or ubiquitin-related polypeptides to their substrates and to poly-ubiquitin-like or poly-ubiquitin-related protein chains.

PGPUB-DOCUMENT-NUMBER: 20040047846

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040047846 A1

TITLE: Ubiquitin promoter in vectors for gene therapy in respiratory tract

PUBLICATION-DATE: March 11, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hyde, Stephen Charles	Oxford		GB	

APPL-NO: 10/ 296261

DATE FILED: May 7, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0013108.6	2000GB-0013108.6	May 30, 2000

PCT-DATA:
APPL-NO: PCT/GB01/02391
DATE-FILED: May 30, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 424/93.21, 424/93.2 , 514/44

ABSTRACT:

The human Ubiquitin C promoter is proposed as a highly advantageous promoter for use in airway gene therapy.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (2):
[0001] The present invention relates to vectors for use in gene therapy, in particular, for example, for directing improved transgene expression for therapeutic purpose in the lung. The vectors of concern include the coding sequence for a therapeutic agent under the control of the human Ubiquitin C (UbC) promoter or a functional analogue of that promoter.

Summary of Invention Paragraph - BSTX (7):
[0005] With a view to finding improved expression vectors for airway gene therapy which will be of clinical benefit, the inventor has turned to investigation of known promoters of human genes having ubiquitous expression in tissues. Investigation was previously reported of the effectiveness of the Elongation Factor 1a (EF1a) promoter in plasmid DNA for directing expression of the firefly luciferase gene in mouse lung after intranasal administration. For comparison, an identical plasmid was used apart from substitution of the EF1a promoter by the conventionally employed immediate early CMV promoter/enhancer.

With the CMV promoter, luciferase expression was maximal after 2 days but essentially undetectable by day 7. While greater persistence of expression of luciferase was observed with the EF1a promoter, reporter gene activity was far lower (7-fold lower at day 2, 38% of day 2 level at day 7 and 23% of day 2 level at day 14; see Abstract 254 of the Proceedings of the 13th Annual North American Cystic Fibrosis Conference, Pediatric Pulmonary Supplement 19, 1994). It has now been found that by substituting the EF1a promoter in the same plasmid vector by the human UbC promoter not only can expression of luciferase in lung comparable to that observed with use of the CMV promoter be achieved but such expression is sustained for a number of weeks. Such expression of a therapeutic agent, e.g. the cystic fibrosis transmembrane conductance regulator gene product in the lungs of cystic fibrosis sufferers, is anticipated to be of clinical benefit.

Summary of Invention Paragraph - BSTX (8):
[0006] The human UbC promoter has previously been shown to direct high level recombinant protein expression in a variety of mammalian cell lines (Wulff et al., FEBS Letters (1990) 261, 101-105; Johansen et al., FEBS Letters (1990) 267, 289-294) and in a wide range of tissues of transgenic mice including lung (Shorpp et al., Nucleic Acid Res. (1996) 24, 1787-1788). However, such studies do not enable direct extrapolation as to whether expression vectors relying on the human UbC promoter for expression of a therapeutic agent, when administered to the airways, will provide a sufficient degree and endurance of expression of the desired therapeutic agent for successful gene therapy.

Summary of Invention Paragraph - BSTX (9):
[0007] Expression vectors comprising the human UbC promoter have been proposed for delivery of therapeutic genes to the central nervous system (WO 98/32869). However, there has been no suggestion that such vectors may be of use in airway gene therapy. Furthermore, the studies reported in WO 98/32869 do not enable direct extrapolation of the success of expression vectors which rely on the human UbC promoter for expression of a therapeutic agent, when administered to the airways.

Summary of Invention Paragraph - BSTX (10):
[0008] The studies reported herein for the first time establish the human UbC promoter as a candidate highly advantageous promoter for gene therapy in the lung.

Summary of Invention Paragraph - BSTX (12):
[0009] In one aspect, the present invention thus provides the use of a vector including a human Ubiquitin C (UbC) promoter or functional analogue thereof operably-linked to a coding sequence for a therapeutic agent in the manufacture of a medicament for use in airway gene therapy, in a human or non-human animal. As indicated above, such airway gene therapy may particularly be for treatment of cystic fibrosis, asthma, emphysema, pulmonary oedema or lung cancer, especially cystic fibrosis.

Summary of Invention Paragraph - BSTX (13):
[0010] In a further aspect, the present invention provides vectors for use in treating cystic fibrosis, emphysema or pulmonary oedema, wherein a human UbC promoter or functional analogue thereof is employed to direct expression of the desired therapeutic agent.

Summary of Invention Paragraph - BSTX (19):
[0015] An expression vector for use in accordance with the invention may include in addition to the human UbC promoter or a functional analogue thereof other control elements conventionally employed in expression vectors operably linked to the coding sequence for the desired therapeutic agent, e.g. a

transcription termination sequence and/or a poly A sequence and/or an enhancer element.

Summary of Invention Paragraph - BSTX (20):
[0016] The human UbC promoter has previously been cloned and may be obtained, for example, by PCR amplification from the known plasmid pUB6/V5-His A (Invitrogen). By functional analogue of that promoter will be understood any promoter which represents a derivative of the human UbC promoter and retains the ability to sustain expression of the luciferase gene from plasmid DNA in mouse lung in vivo for at least a period of weeks, e.g at least 4 weeks, preferably at least 8 weeks. Preferably such a functional analogue will achieve expression in such an animal model comparable to the maximum obtainable by substitution of the human UbC promoter, e.g at least 50%, more preferably at least 70 to 100% of the maximum expression obtainable with the human UbC promoter. A functional analogue of the human UbC promoter may be an equivalent gene promoter from a non-human mammalian species. It may be a modified human or non-human UbC promoter having one or more base pair substitutions and/or incorporating one or more modified bases.

Summary of Invention Paragraph - BSTX (22):
[0018] For treatment of emphysema, the human UbC promoter or functional analogue thereof will direct expression of human alpha-1 anti-trypsin or an analogue thereof which is capable of producing a functionally equivalent therapeutic effect. Isolation of the cDNA for human alpha-1 anti-trypsin has also previously been described (see GenBank Accession no. NM-00295 and Ciliberto et al., Cell-specific expression of a transfected human alpha 1-anti-trypsin gene, Cell (1985) 41, 531-540).

Summary of Invention Paragraph - BSTX (23):
[0019] As hereinbefore indicated, vectors for use in accordance with the invention are also proposed for treatment of pulmonary oedema In this case, the human UbC promoter or functional analogue thereof may direct expression of the human sodium-potassium-adenosinetriphosphatase enzyme or an analogue of that enzyme which produces the desired therapeutic effect. cDNAs encoding both chains of the human sodium-potassium-adenosin- etriphosphatase have also previously been cloned and sequenced (see GenBank accession nos. AH001423 (alpha subunit and U50743 (gamma subunit); see also Sverdlov et al., The family of human Na.sup.+K.sup.+-ATPase: No less than five genes and/or pseudogenes related to the alpha-subunit, FEBS Lett. (1987) 217, 275-278).

Summary of Invention Paragraph - BSTX (32):
[0028] Vectors for use in accordance with the invention to treat lung cancer may rely on a human UbC promoter or functional analogue thereof to direct expression in the lungs of various therapeutic agents previously proposed for treatment of cancers, including, for example, preferably prodrug-converting enzymes. By prodrug-converting enzyme will be understood a gene product which activates a compound with little or no cytotoxicity into a toxic product. Various prodrug activation strategies employing viral vectors have previously been proposed for cancer treatment (see, for example, Published International Application no. WO 95/07994 and EP-B 0 702 084 of Chiron Corp.) and may be adopted in the lungs by provision of a vector in accordance with the present invention together with the appropriate prodrug. Thus, for example, a vector for use in lung cancer therapy may preferably be constructed such that a human UbC promoter or functional analogue thereof directs expression of a viral thymidine kinase, e.g. Herpes simplex virus thymidine kinase. For prodrug-activation therapy, such an enzyme is employed together with a purine or pyrimidine analogue, e.g. ganciclovir, which is phosphorylated by the viral thymidine kinase to a toxic triphosphate form. Examples of other prodrug-converting enzymes which may be advantageously expressed from a human

UbC promoter in the lungs for prodrug activation therapy of lung cancer include:

Summary of Invention Paragraph - BSTX (40):
[0036] In a still further aspect, the present invention provides a method of treating an airway- or lung-associated disease, e.g. a disease selected from the group consisting of cystic fibrosis, asthma, pulmonary oedema, emphysema and lung cancer which comprises administering to the airways or lung a vector including a human UbC promoter or functional analogue thereof as hereinbefore described. Suitable dosages for administration of the vector may be determined by appropriate trial. A dosage of 10 to 100 mg DNA may, for example, be found suitable.

Brief Description of Drawings Paragraph - DRTX (2):
[0038] FIG. 1 shows the expression of firefly luciferase in human embryonic kidney 293T cells in vitro, two days after transfection with the plasmids PCIKLux, pUbLux and pEFLux containing the CMV immediate early promoter/enhancer, the human UbC promoter and the human elongation factor 1 alpha promoter respectively, operably-linked to a luciferase coding sequence. Reporter gene expression is given as a percentage of the average reporter gene activity obtained with the CMV promoter.

Brief Description of Drawings Paragraph - DRTX (5):
[0041] FIG. 4 shows the expression of firefly luciferase in mouse lung following airway administration of the plasmid pCIKLux or a Sleeping Beauty (SB) vector plasmid DNA containing the human UbC promoter (SB Ub) or the CMV immediate early promoter/enhancer (SB CMV) operably linked to a luciferase coding sequence. Both integrating and non-integrating SB vectors were used.

Detail Description Paragraph - DETX (3):
[0043] The effectiveness of the CMV immediate early, human elongation factor 1 alphas and human UbC promoters in directing protein expression in cells grown in culture was compared using a transient plasmid transfection. Plasmid expression vectors were employed containing the human UbC promoter (pUbLux), the CMV immediate early promoter/enhancer (pCIKLux) or the human elongation factor 1 promoter (pEFLux) each directing the expression of a firefly luciferase gene.

Detail Description Paragraph - DETX (5):
[0045] The plasmids pUbLux, pCIKLux and pEFLux were constructed starting from the commercially available eukaryotic expression plasmid pCI (Promega, Southampton, U.K.). A PCR fragment containing the human UbC promoter was obtained by PCR amplification from pUB6/V5-His A (Invitrogen, Gronigen, Netherlands). A PCR fragment containing the human Elongation Factor 1 alpha promoter was obtained by PCR amplification from pEF1/V5-His A (Invitrogen, Gronigen, Netherlands).

Detail Description Paragraph - DETX (10):
[0050] A plasmid designated pUb was constructed by replacing the BglII-NheI CMV promoter restriction fragment in pCI with a 1218 bp BglII-NheI restriction fragment (numbering includes the entire restriction enzyme recognition sequences) including the human UbC promoter, exon 1, intron 1 and the 5' 2 bp of exon 2 (bases -333 to +877 ggcctc . . . ttagac relative to the transcription start site) isolated from plasmid pKSMUb.

Detail Description Paragraph - DETX (25):
[0065] CMV promoter mediated reporter gene expression following transient

transfection of 293T cells cultured in vitro was significantly greater than expression directed by either the human UbC or human elongation factor 1 alpha promoters (FIG. 1).

Detail Description Paragraph - DETX (27):
[0066] Comparison of the CMV Immediate Early Promoters the Human Elongation Factor 1 Alpha Promoter and the Human UbC Promoter for Directing Protein Expression in the Lungs

Detail Description Paragraph - DETX (28):
[0067] The effectiveness of the human UbC promoter in directing protein expression in the lungs was studied in a mouse model system employing a plasmid expression vector vector (pUbLux) containing the human UbC promoter directing the expression of a firefly luciferase gene. As a comparison, the same vector was employed but with the human UbC promoter substituted by either the CMV immediate early promoter/enhancer (pCIKLux), or the human Elongation factor 1 alpha promoter (pEFLux). The plasmids were constructed as described in Example 1.

Detail Description Paragraph - DETX (34):
[0073] CMV promoter mediated reporter gene expression after 2 days was significantly greater than expression directed by either the human UbC or human elongation factor 1 alpha promoters (FIG. 2).

Detail Description Paragraph - DETX (37):
[0076] In contrast, although the human UbC promoter directed a relatively low level of reporter gene expression at day 2 after dosing, expression of luciferase from pUbLux was found to increase to day 14 and was subsequently sustained at a level similar to the peak expression levels observed with the CMV promoter for up to at least 8 weeks from initial dosing. As shown in FIG. 1, reporter gene expression directed by the human UbC promoter, albeit at a level lower than the maximum obtained with the CMV promoter, was observed even after 26 weeks (182 days) (FIG. 3). Thus, the human UbC promoter directs persistent and abundant reporter gene expression in mouse lung following naked plasmid DNA mediated gene transfer.

Detail Description Paragraph - DETX (40):
[0078] The effectiveness of the human UbC promoters in directing protein expression in the lungs was studied using a mouse model system employing a Sleeping Beauty non-viral integrating gene transfer vector (Ivics, Z., Hackett, P. B., Plasterk, R. H. and Izsvak, Z. Cell 1997; 91: 501-510) containing the human UbC promoter directing the expression of a firefly luciferase gene (SB Ub). As a comparison, the same vector was employed but with the human UbC promtoer substituted by the CMV immediate early promoter/enhancer (SB CMV).

Detail Description Paragraph - DETX (45):
[0083] The plasmid pTB/HUbLux was constructed by inserting the 3139 bp BglII-BamHI restriction fragment (numbering including the entire restriction enzyme recognition sequences) from pUbLux containing the human UbC promoter, exon 1, intron 1 5' of exon 2, consensus Kozak translation signal, firefly luciferase gene, and SV40 late polyadenylation signal into the unique BglII site of pTB/H.

Detail Description Paragraph - DETX (56):
[0094] The duration of CMV mediated airway luciferase reporter gene expression was similar if mediated through plasmid DNA (pCIKLux), a non-integrating Sleeping Beauty transposon (SB CMV Non Integrating) or a Sleeping Beauty trasposon with the capacity to integrate into the airway cell genome (SB CMV Integrating). However, as in the context of plasmid DNA (FIG.

3) the duration of human UbC promoter expression was significantly greater than that directed by the CMV immediate early promoter/enhancer in the context of the Sleeping Beauty transposon gene transfer system (FIG. 4). Furthermore, reporter gene expression directed by a non-integrating Sleeping Beauty transposon (SB Ub Non Integrating) was significantly less than a Sleeping Beauty transposon with the capacity to integrate into the airway cell genome (SB Ub Integrating) (p=0.0374 at day 84 post dosing, Mann Whitney Non-Parametric Statistical Analysis).

Detail Description Paragraph - DETX (59):

[0096] A first vector was made in which the human UbC promoter drives expression of the human cystic fibrosis transmembrane conductance regulator (CFTR) gene (vector pUbCFTR) by replacing the NheI-NotI fragment of pUbLux containing the luciferase coding sequence with the human CFTR cDNA. The NheI-NotI CFTR cDNA fragment was isolated from pCIKCFTR. This plasmid was constructed by inserting a KpnI-NotI fragment containing the entire CFTR cDNA from the plasmid pTRIAL10-CFTR2 into the KpnI-NotI sites in the polylinker of pCI. The construction of plasmid pTRIAL10-CFTR2 has previously been described in Gill et al., Gene Therapy (1997) 4, 199-209.

Detail Description Paragraph - DETX (64):

[0100] An alternative plasmid vector for use in Cystic Fibrosis patients can be obtained by inserting the human CFTR gene into the plasmid pVAX (Invitrogen) in which the CMV promoter has also been substituted by the human UbC promoter. Plasmid pVAX is a vector constructed to be consistent with the above-noted FDA document and contains in addtion to a CMV promoter, the origin of replication from plasmid pMB1 and a kanamycin resistance gene.

Claims Text - CLTX (1):

1. Use of a vector including a human Ubiquitin C (UbC) promoter or functional analogue thereof operably-linked to a coding sequence for a therapeutic agent in the manufacture of a medicament for use in airway gene therapy.

Claims Text - CLTX (2):

2. Use of a vector according to claim 1 wherein the human UbC promoter is operably-linked to a protein coding sequence.

PGPUB-DOCUMENT-NUMBER: 20040043388

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040043388 A1

TITLE: Three hybrid assay system

PUBLICATION-DATE: March 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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APPL-NO: 10/ 234985

DATE FILED: September 3, 2002

RELATED-US-APPL-DATA:

child 10234985 A1 20020903

parent continuation-in-part-of 10091177 20020304 US PENDING

non-provisional-of-provisional 60272932 20010302 US

non-provisional-of-provisional 60278233 20010323 US

non-provisional-of-provisional 60329437 20011015 US

US-CL-CURRENT: 435/6, 435/7.1 , 530/317 , 530/350 , 536/123 , 536/23.1 , 540/200 , 546/1 , 552/200 , 552/500 , 552/653 , 556/118

ABSTRACT:

The invention provides compositions and methods for isolating ligand binding polypeptides for a user-specified ligand, and for isolating small molecule ligands for a user-specified target polypeptide using an improved class of hybrid ligand compounds.

REFERENCE TO RELATED APPLICATIONS

[0001] This application is a "continuation in part (CIP)" application of U.S. Ser. No. 10/091,177, filed on Mar. 4, 2002, which claims priority to U.S. Provisional applications No. 60/272,932, filed on Mar. 2, 2001; No. 60/278,233, filed on Mar. 23, 2001; and No. 60/329,437, filed on Oct. 15, 2001, the specifications of which are hereby incorporated by reference in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (75):
[0200] The "ubiquitins" are a class of proteins found in all eukaryotic cells. The ubiquitin polypeptide is characterized by a carboxy-terminal

glycine residue that is activated by ATP to a high-energy thiol-ester intermediate in a reaction catalyzed by a ubiquitin-activating enzyme (E1). The activated ubiquitin is transferred to a substrate polypeptide via an isopeptide bond between the activated carboxy-terminus of ubiquitin and the epsilon-amino group of (a) lysine residue(s) in the protein substrate. This transfer requires the action of ubiquitin conjugating enzymes such as E2 and, in some instances, E3 activities. The ubiquitin modified substrate is thereby altered in biological function, and, in some instances, becomes a substrate for components of the ubiquitin-dependent proteolytic machinery which includes both UBP enzymes as well as proteolytic proteins which are subunits of the proteasome. As used herein, the term "ubiquitin" includes within its scope all known as well as unidentified eukaryotic ubiquitin homologs of vertebrate or invertebrate origin which can be classified as equivalents of human ubiquitin. Examples of ubiquitin polypeptides as referred to herein include the human ubiquitin polypeptide which is encoded by the human ubiquitin encoding nucleic acid sequence (GenBank Accession Numbers: U49869, X04803). Equivalent ubiquitin polypeptide encoding nucleotide sequences are understood to include those sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants; as well as sequences which differ from the nucleotide sequence encoding the human ubiquitin coding sequence due to the degeneracy of the genetic code. Another example of a ubiquitin polypeptide as referred to herein is murine ubiquitin which is encoded by the murine ubiquitin encoding nucleic acid sequence (GenBank Accession Number: X51730). It will be readily apparent to the person skilled in the art how to modify the methods and reagents provided by the present invention to the use of ubiquitin polypeptides other than human ubiquitin.

Detail Description Paragraph - DETX (79):

[0204] The term "ubiquitin conjugation machinery" as used herein refers to a group of proteins which function in the ATP-dependent activation and transfer of ubiquitin to substrate proteins. The term thus encompasses: El enzymes, which transform the carboxy-terminal glycine of ubiquitin into a high energy thiol intermediate by an ATP-dependent reaction; E2 enzymes (the UBC genes), which transform the E1-S.about.Ubiquitin activated conjugate into an E2-S.about.Ubiquitin intermediate which acts as a ubiquitin donor to a substrate, another ubiquitin moiety (in a poly-ubiquitination reaction), or an E3; and the E3 enzymes (or ubiquitin ligases) which facilitate the transfer of an activated ubiquitin molecule from an E2 to a substrate molecule or to another ubiquitin moiety as part of a polyubiquitin chain. The term "ubiquitin conjugation machinery", as used herein, is further meant to include all known members of these groups as well as those members which have yet to be discovered or characterized but which are sufficiently related by homology to known ubiquitin conjugation enzymes so as to allow an individual skilled in the art to readily identify it as a member of this group. The term as used herein is meant to include novel ubiquitin activating enzymes which have yet to be discovered as well as those which function in the activation and conjugation of ubiquitin-like or ubiquitin-related polypeptides to their substrates and to poly-ubiquitin-like or poly-ubiquitin-related protein chains.

PGPUB-DOCUMENT-NUMBER: 20040043386

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040043386 A1

TITLE: Methods and compositions for functional ubiquitin assays

PUBLICATION-DATE: March 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Wong, Brian	Los Altos	CA	US	
Bennett, Mark	Moraga	CA	US	
Parlati, Frank	San Francisco	CA	US	

APPL-NO: 10/ 232951

DATE FILED: August 30, 2002

US-CL-CURRENT: 435/6, 435/456 , 435/7.2

ABSTRACT:

The present attention is directed to compositions and methods for performing functional assays to determine the physiological role of ubiquitin agents and ubiquitin moieties.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (12):
[0029] FIGS. 11A and 11B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, Ubc8.

Brief Description of Drawings Paragraph - DRTX (13):
[0030] FIGS. 12A and 12B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, UbcH9.

Brief Description of Drawings Paragraph - DRTX (14):
[0031] FIGS. 13A and 13B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, Ubc12.

Brief Description of Drawings Paragraph - DRTX (16):
[0033] FIGS. 15A and 15B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, UbcH6.

Brief Description of Drawings Paragraph - DRTX (19):
[0036] FIGS. 18A and 18B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, Ubc13.

PGPUB-DOCUMENT-NUMBER: 20040018625

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040018625 A1

TITLE: Inducible methods for repressing gene function

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Moqtaderi, Zarmik	Boston	MA	US	

APPL-NO: 10/ 350799

DATE FILED: January 24, 2003

RELATED-US-APPL-DATA:

child 10350799 A1 20030124

parent division-of 09508400 20000919 US GRANTED

parent-patent 6576469 US

child 09508400 20000919 US

parent a-371-of-international PCT/US98/19026 19980910 WO PENDING

non-provisional-of-provisional 60058474 19970910 US

US-CL-CURRENT: 435/455, 514/152

ABSTRACT:

Methods for the rapid repression of gene function in eucaryotic cells are disclosed including inducible means for both shutting down a targeted gene's transcription and rapidly removing a targeted gene's polypeptide product.

---------- KWIC ---------

Detail Description Paragraph - DETX (41):

[0048] The term "ubiquitin" as used herein refers to an abundant 76 amino acid residue polypeptide that is found in all eukaryotic cells. The ubiquitin polypeptide is characterized by a carboxy-terminal glycine residue that is activated by ATP to a high-energy thiol-ester intermediate in a reaction catalyzed by a ubiquitin-activating enzyme (E1). The activated ubiquitin is transferred to a substrate polypeptide via an isopeptide bond between the activated carboxy-terminus of ubiquitin and the epsilon-amino group of a lysine residue(s) in the protein substrate. This transfer requires the action of ubiquitin conjugating enzymes such as E2 and, in some instances, E3 activities. The ubiquitin modified substrate is thereby altered in biological function, and, in some instances, becomes a substrate for components of the ubiquitin-dependent proteolytic machinery which includes both ubiquitin isopeptidase enzymes as well as proteolytic proteins which are subunits of the

proteasome. As used herein, the term "ubiquitin" includes within its scope all known as well as unidentified eukaryotic ubiquitin homologs of vertebrate or invertebrate origin. Examples of ubiquitin polypeptides as referred to herein include the human ubiquitin polypeptide which is encoded by the human ubiquitin encoding nucleic acid sequence (GenBank Accession Numbers: U49869, X04803) as well as all equivalents. Equivalent ubiquitin polypeptide encoding nucleotide sequences are understood to include those sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants; as well as sequences which differ from the nucleotide sequence encoding the human ubiquitin coding sequence shown in SEQ ID NO. 2, due to the degeneracy of the genetic code. Another example of a ubiquitin polypeptide as referred to herein is murine ubiquitin which is encoded by the murine ubiquitin encoding nucleic acid sequence (GenBank Accession Number: X51730).

Detail Description Paragraph - DETX (46):

[0053] The term "ubiquitin conjugation machinery" as used herein refers to a group of proteins which function in the ATP-dependent activation and transfer of ubiquitin to substrate proteins. The term thus encompasses: E1 enzymes, which transform the carboxy-terminal glycine of ubiquitin into a high energy thiol intermediate by an ATP-dependent reaction; E2 enzymes (the UBC genes), which transform the E1-S.about.Ubiquitin activated conjugate into an E2-S.about.Ubiquitin intermediate which acts as a ubiquitin donor to a substrate, another ubiquitin moiety (in a poly-ubiquitination reaction), or an E3; and the E3 enzymes (or ubiquitin ligases) which facilitate the transfer of an activated ubiquitin molecule from an E2 to a substrate molecule or to another ubiquitin moiety as part of a polyubiquitin chain. The term "ubiquitin conjugation machinery", as used herein, is further meant to include all known members of these groups as well as those members which have yet to be discovered or characterized but which are sufficiently related by homology to known ubiquitin conjugation enzymes so as to allow an individual skilled in the art to readily identify it as a member of this group. The term as used herein is meant to include novel ubiquitin activating enzymes which have yet to be discovered as well as those which function in the activation and conjugation of ubiquitin-like or ubiquitin-related polypeptides to their substrates and to poly-ubiquitin-like or polyubiquitin-related protein chains.

PGPUB-DOCUMENT-NUMBER: 20040014100

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040014100 A1

TITLE: In vivo production of cyclic peptides for inhibiting protein-protein interaction

PUBLICATION-DATE: January 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Bennett, Mark K.	Moraga		US	

APPL-NO: 10/ 422536

DATE FILED: April 23, 2003

RELATED-US-APPL-DATA:

child 10422536 A1 20030423

parent continuation-of 10232758 20020830 US PENDING

child 10232758 20020830 US

parent continuation-in-part-of 09800770 20010306 US PENDING

non-provisional-of-provisional 60187130 20000306 US

US-CL-CURRENT: 435/6, 435/7.2

ABSTRACT:

The present invention relates to methods and compositions utilizing inteins to generate libraries of cyclic peptides in vivo. The prevent invention also relates to methods for inhibiting protein-protein interaction.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (66):

[0078] FIGS. 27A and 27B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, Ubc8.

Brief Description of Drawings Paragraph - DRTX (67):

[0079] FIGS. 28A and 28B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, UbcH9.

Brief Description of Drawings Paragraph - DRTX (68):

[0080] FIGS. 29A and 29B show the nucleic acid sequence and amino acid

sequence, respectively, of a human E2, Ubc12.

Brief Description of Drawings Paragraph - DRTX (70):

[0082] FIGS. 31A and 31B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, UbcH6.

Brief Description of Drawings Paragraph - DRTX (73):

[0085] FIGS. 34A and 34B show the nucleic acid sequence and amino acid sequence, respectively, of a human E2, Ubc13.

PGPUB-DOCUMENT-NUMBER: 20030228617

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030228617 A1

TITLE: Method for predicting autoimmune diseases

PUBLICATION-DATE: December 11, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Olsen, Nancy J.	Nashville	TN	US	

APPL-NO: 10/ 439388

DATE FILED: May 16, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60381055 20020516 US

US-CL-CURRENT: 435/6, 435/91.2

ABSTRACT:

The presently claimed subject matter provides a method for detecting an autoimmune disorder in a subject by obtaining a biological sample from the subject; determining expression levels of at least two genes in the biological sample; and comparing the expression level of each gene with a standard, wherein the comparing detects the presence of an autoimmune disorder in the subject. Also provided are compositions and kits for carrying out the methods of the presently claimed subject matter.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is based on and claims priority to U.S. Provisional Application Serial No. 60/381,055, filed May 16, 2002, herein incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention - Table CWU - BSTL (1):

1 Table of Abbreviations 6-JOE - 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein, succinimidyl ester aaRNA - amplified antisense RNA Ags - antigens AP3S2 - adaptor-related protein complex 3, sigma 2 subunit ASL - argininosuccinate lyase BMP8 - bone morphogenetic protein 8 (osteogenic protein 2) BPHL - biphenyl hydrolase-like (serine hydrolase; breast epithelial mucin-associated antigen) BRCA1 - breast cancer 1, early onset, transcript variant BRCA1a CASP6 - caspase 6 CDH1 - cadherin 1, type 1, E-cadherin (epithelial) CDKN1B - cyclin-dependent kinase inhibitor 1B cDNA - complementary DNA CYB5-M - cytochrome b5 outer mitochondrial membrane precursor DEPC - diethylpyrocarbonate DIPA - hepatitis delta antigen-interacting protein A DMARDs - disease-modifying anti-rheumatic drugs DNAJA1 - DnaJ homolog, subfamily A, member 1 EPB72 - erythrocyte membrane protein band 7.2 (stomatin) EST - expressed sequence tag FITC - fluorescein

isothiocyanate GMBS - gamma-maleimidobutyryloxy-succimide GNB5 - human guanine nucleotide binding protein, beta 5 GUCY1B3 - guanylate cyclase 1, soluble, beta 3 HSJ2 - heat shock protein, DNAJ-like 2 IDDM - insulin-dependent (type 1) diabetes mellitus IFN - interferon LabMAP - Laboratory Multiple Analyte Profiling LIF - leukemia inhibitory factor LLGL2 - lethal giant larvae homolog 2 MAN1A1 - mannosidase, alpha, class 1A, member 1 MMP17 - matrix metalloproteinase 17 MS - multiple sclerosis MYO1C - myosin I C NSAIDs - nonsteroidal anti-inflammatory drugs ORC1L - origin recognition complex, subunit 1-like PCR - polymerase chain reaction PMBC - peripheral blood mononuclear cell(s) RA - rheumatoid arthritis RAPD - rapid amplification of polymorphic DNA ROCK - Random Oligonucleotide Construction Kit RTN4 - reticulon 4 RT-PCR - reverse transcription PCR SC65 - synaptonemal complex protein 65 SD - standard deviation(s) SIP1 - survival of motor neuron protein interacting protein 1 SISPA - Sequence-Independent, Single-Primer Amplification SLC16A4 - solute carrier family 16, member 4 SLE - systemic lupus erythematosus SSP29 - silver-stainable protein 29, also called acidic (leucine-rich) nuclear phosphoprotein 32 family, member B STOM - alternate abbreviation for stomatin SUDD - human sudD suppressor of bimD6 homolog (SUDD) from Aspergillus nidulans, transcript variant 1 TAF11 - TATA box binding protein- associated factor 11 TAF2I - TAF11 RNA polymerase II, TATA box binding protein-associated factor, 28 kilodalton TBP - TATA box binding protein TGM2 - transglutaminase 2 TNF-.alpha. - tumor necrosis factor alpha TNFAIP2 - tumor necrosis factor, alpha-induced protein 2 TP53 - human tumor protein p53 (Li-Fraumeni syndrome) TXK - TXK tyrosine kinase UBE2G2 - ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast)

Detail Description Paragraph - DETX (34):

[0058] SEQ ID NOs: 65 and 66 are the nucleic acid sequences of a partial cDNA and a full-length cDNA, respectively, corresponding to the human ubiquitin-conjugating enzyme E2G 2 (UBC7 homolog, yeast; UBE2G2) gene (GenBank Accession Nos. AA443634 and NM.sub.--003343).

Detail Description Table CWU - DETL (1):

3TABLE 1 Genes Used in the Equation Gene SEQ ID Symbol Gene Name NOs: TGM2 transglutaminase 2 1, 2 SSP29 silver-stainable protein 29 3, 4 TAF2I TAF11 RNA polymerase II, TATA box 5, 6 binding protein-associated factor, 28 kilodalton LLGL2 lethal giant larvae homolog 2 7, 8 TNFAIP2 tumor necrosis factor, alpha-induced protein 9, 10 2 SIP1 survival of motor neuron protein interacting 11, 12 protein 1 BPHL biphenyl hydrolase-like 13, 14 TP53 human tumor protein p53 15, 16 DIPA hepatitis delta antigen-interacting protein A 17, 18 ASL argininosuccinate lyase 19, 20 GNB5 human guanine nucleotide binding protein, 21, 22 beta 5 MAN1A1 mannosidase, alpha, class 1A, member 1 23, 24 -- EST 25, 26 LOC51643 CGI-119 protein 27, 28 BMP8 bone morphogenetic protein 8 29, 30 -- human mRNA for cytochrome b5, partial 31, 32 coding sequence ORC1L origin recognition complex, subunit 1-like 33, 34 -- EST 35, 36 CDH1 cadherin 1, type 1, E-cadherin 37, 38 SUDD human sudD suppressor of bimD6 homolog 39, 40 (SUDD) EPB72 erythrocyte membrane protein band 7.2 41, 42 CDKN1B cyclin-dependent kinase inhibitor 1B 43, 44 CASP6 caspase 6 45, 46 TXK TXK tyrosine kinase 47, 48 MYO1C myosin IC 49, 50 -- EST 51, 52 HSJ2 heat shock protein, DNAJ-like 2 53, 54 BRCA1 breast cancer 1, early onset, transcript 55, 56 variant BRCA1a GUCY1B3 guanylate cyclase 1, soluble, beta 3 57, 58 AP3S2 adaptor-related protein complex 3, sigma 2 59, 60 subunit -- EST 61, 62 SC65 synaptonemal complex protein 65 63, 64 UBE2G2 ubiquitin-conjugating enzyme E2G 2 65, 66 SLC16A4 solute carrier family 16, member 4 67, 68 MMP17 matrix metalloproteinase 17 69, 70

PGPUB-DOCUMENT-NUMBER: 20030224376

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030224376 A1

TITLE: Novel human transferase family members and uses thereof

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

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Williamson, Mark	Saugus	MA	US	
Leiby, Kevin R.	Natick	MA	US	
Kapeller-Libermann, Rosana	Chestnut Hill	MA	US	
Olandt, Peter J.	Newton	MA	US	
MacBeth, Kyle J.	Boston	MA	US	
Rudolph-Owen, Laura A.	Jamaica Plain	MA	US	
Tsai, Fong-Ying	Newton	MA	US	
Hunter, John J.	Somerville	MA	US	

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DATE FILED: June 27, 2002

RELATED-US-APPL-DATA:

child 10184648 A1 20020627

parent continuation-in-part-of 09815028 20010322 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 09801220 20010307 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 09816714 20010323 US ABANDONED

child 10184648 A1 20020627

parent continuation-in-part-of 09844948 20010427 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 09861164 20010518 US ABANDONED

child 10184648 A1 20020627

parent continuation-in-part-of 09883060 20010615 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 09962678 20010925 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 09973457 20011009 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 10072285 20020208 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 09817910 20010326 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 09842528 20010425 US ABANDONED

child 10184648 A1 20020627

parent continuation-in-part-of 09882836 20010615 US PENDING

child 10184648 A1 20020627

parent continuation-in-part-of 09882872 20010615 US ABANDONED

non-provisional-of-provisional 60191964 20000324 US

non-provisional-of-provisional 60187456 20000307 US

non-provisional-of-provisional 60191865 20000324 US

non-provisional-of-provisional 60200604 20000428 US

non-provisional-of-provisional 60205408 20000519 US

non-provisional-of-provisional 60212079 20000615 US

non-provisional-of-provisional 60235044 20000925 US

non-provisional-of-provisional 60238849 20001006 US

non-provisional-of-provisional 60267494 20010208 US

non-provisional-of-provisional 60192092 20000324 US

non-provisional-of-provisional 60199500 20000425 US

non-provisional-of-provisional 60211730 20000615 US

non-provisional-of-provisional 60212077 20000615 US

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US01/09358	2001WO-PCT/US01/09358	March 22, 2001
WO	PCT/US01/07269	2001WO-PCT/US01/07269	March 7, 2001
WO	PCT/US01/09468	2001WO-PCT/US01/09468	March 23, 2001
WO	PCT/US01/13805	2001WO-PCT/US01/13805	April 27, 2001
WO	PCT/US01/16292	2001WO-PCT/US01/16292	May 18, 2001
WO	PCT/US01/19138	2001WO-PCT/US01/19138	June 15, 2001
WO	PCT/US01/29963	2001WO-PCT/US01/29963	September 25, 2001
WO	PCT/US02/03736	2002WO-PCT/US02/03736	February 8, 2002
WO	PCT/US01/09633	2001WO-PCT/US01/09633	March 26, 2001

WO	PCT/US01/40607	2001WO-PCT/US01/40607	April 25, 2001
WO	PCT/US01/19543	2001WO-PCT/US01/19543	June 15, 2001
WO	PCT/US01/19153	2001WO-PCT/US01/19153	June 15, 2001

US-CL-CURRENT: 435/6, 424/144.1 , 435/320.1 , 435/325 , 435/69.1 , 514/1 , 514/12 , 514/7 , 530/350 , 536/23.2

ABSTRACT:

The invention provides isolated nucleic acids molecules, designated 33877, 47179, 26886, 25552, 32132, 32244, 23680, 32624, 47174, 60491, 46743, 27417, 27960, 32252, and 53320 nucleic acid molecules, which encode novel human transferase family members. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing 33877, 47179, 26886, 25552, 32132, 32244, 23680, 32624, 47174, 60491, 46743, 27417, 27960, 32252, or 53320 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a 33877, 47179, 26886, 25552, 32132, 32244, 23680, 32624, 47174, 60491, 46743, 27417, 27960, 32252, or 53320 gene has been introduced or disrupted. The invention still further provides isolated 33877, 47179, 26886, 25552, 32132, 32244, 23680, 32624, 47174, 60491, 46743, 27417, 27960, 32252, or 53320 proteins, fusion proteins, antigenic peptides and anti-33877, 47179, 26886, 25552, 32132, 32244, 23680, 32624, 47174, 60491, 46743, 27417, 27960, 32252, or 53320 antibodies. Diagnostic methods utilizing compositions of the invention are also provided.

RELATED APPLICATIONS

[0001] This application is a continuation-in-part and claims priority to U.S. application Ser. No. 09/815,028, filed Mar. 22, 2001, and International Application Serial No. PCT/US01/09358, filed Mar. 22, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/191,964, filed Mar. 24, 2000; and U.S. application Ser. No. 09/801,220, filed Mar. 07, 2001, and International Application Serial No. PCT/US01/07269, filed Mar. 07, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/187,456, filed Mar. 07, 2000; and U.S. application Ser. No. 09/816,714, filed Mar. 23, 2001, and International Application Serial No. PCT/US01/09468, filed Mar. 23, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/191,865, filed Mar. 24, 2000; and U.S. application Ser. No. 09/844,948, filed Apr. 27, 2001, and International Application Serial No. PCT/US01/13805, filed Apr. 27, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/200,604, filed Apr. 28, 2000; and U.S. application Ser. No. 09/861,164, filed May 18, 2001, and International Application Serial No. PCT/US01/16292, filed May 18, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/205,408, filed May 19, 2000; and U.S. application Ser. No. 09/883,060, filed Jun. 15, 2001, and International Application Serial No. PCT/US01/19138, filed Jun. 15, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/212,079, filed Jun. 15, 2000; and U.S. application Ser. No. 09/962,678, filed Sep. 25, 2001, and International Application Serial No. PCT/US01/29963, filed Sep. 25, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/235,044, filed Sep. 25, 2000; and U.S. application Ser. No. 09/973,457, filed Oct. 09, 2001, which claims the benefit of U.S. Provisional Application Serial No. 60/238,849, filed Oct. 06, 2000; and U.S. application Ser. No. 10/072,285, filed Feb. 08, 2002, and International Application Serial No. PCT/US02/03736, filed Feb. 08, 2002, which claim the benefit of U.S. Provisional Application Serial No. 60/267,494, filed Feb. 08, 2001; and U.S. application Ser. No. 09/817,910, filed Mar. 26, 2001, and International Application Serial No. PCT/US01/09633, filed Mar. 26, 200 1, which claim the benefit of U.S. Provisional Application Serial No. 60/192,092, filed Mar. 24, 2000; and U.S.

application Ser. No. 09/842,528, filed Apr. 25, 2001, and International Application Serial No. PCT/US01/40607, filed Apr. 25, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/199,500, filed Apr. 25, 2000; and U.S. application Ser. No. 09/882,836, filed Jun. 15, 2001, and International Application Serial No. PCT/US01/19543, filed Jun. 15, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/211,730, filed Jun. 15, 2000; and U.S. application Ser. No. 09/882,872, filed Jun. 15, 2001, and International Application Serial No. PCT/US01/19153, filed Jun. 15, 2001, which claim the benefit of U.S. Provisional Application Serial No. 60/212,077, filed Jun. 15, 2000, the contents of which are incorporated herein by reference.

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Brief Description of Drawings Paragraph - DRTX (66):

[0103] FIGS. 65A-65B depict alignment of the ubiquitin-conjugating enzyme domain of human 27960 with a consensus amino acid sequence derived from hidden Markov models using PFAM (UQ_con) and SMART (ubc.sub.--7) programs, respectively. In FIG. 65A, the upper sequence is the consensus amino acid sequence (SEQ ID NO: 75), while the lower amino acid sequence corresponds to amino acids 1 to 148 of SEQ ID NO: 73. In FIG. 65B, the upper sequence is the consensus amino acid sequence (SEQ ID NO: 76), while the lower amino acid sequence corresponds to amino acids 6 to 151 of SEQ ID NO: 73.

Detail Description Paragraph - DETX (4414):

[4233] The present invention is based, in part, on the discovery of a novel human ubiquitin conjugating enzyme, referred to herein as "27960". The nucleotide sequence of a cDNA encoding 27960 is shown in SEQ ID NO: 72, and the amino acid sequence of a 27960 polypeptide is shown in SEQ ID NO: 73. In addition, the nucleotide sequences of the coding region are depicted in SEQ ID NO: 74.

Detail Description Paragraph - DETX (4442):

[4260] Human 27960 contains the following regions or other structural features: a ubiquitin-conjugating enzyme domain (PFAM Accession PF00179) located at about amino acid residues 1 to 148 of SEQ ID NO: 73; two predicted Protein Kinase C sites (PS00005) at about amino acids 61 to 63 and 118 to 120 of SEQ ID NO: 73; three predicted Casein Kinase II sites (PS00006) located at about amino acids 24 to 27, 47 to 50, and 118 to 121 of SEQ ID NO: 73; one tyrosine kinase phosphorylation site (PS00007) at amino acids 123 to 131 of SEQ ID NO: 73, and two predicted N-myristylation sites (PS00008) from about amino acids 22 to 27 and 43 to 48 of SEQ ID NO: 73.

Detail Description Paragraph - DETX (4445):

[4263] The 27960 protein contains a significant number of structural characteristics in common with members of the ubiquitin-conjugating enzyme family. The term "family" when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-human origin, e.g., rat or mouse proteins. Members of a family can also have common functional characteristics.

Detail Description Paragraph - DETX (4448):

[4266] Preferably, the ubiquitin-conjugating enzyme domain includes an amino acid sequence of about 100 to 200 amino acids, preferably 130 to 160, more preferably about 145 to 148 amino acid residues in length and having a bit score for the alignment of the sequence to the ubiquitin-conjugating enzyme domain (HMM) of at least 100, more preferably 120, and most preferably 125 to 140. The ubiquitin-conjugating enzyme domain (HMM) has been assigned the PFAM Accession No. PF00179 (http;//genome.wust1.edu/Pfam/.html). An alignment of the ubiquitin-conjugating enzyme domain (amino acids 1 to 148 of SEQ ID NO: 73) of human 27960 with a consensus amino acid sequence derived from hidden Markov models are depicted in FIGS. 65A and 65B.

Detail Description Paragraph - DETX (4449):

[4267] In a preferred embodiment, 27960 polypeptide or protein has a "ubiquitin-conjugating enzyme domain" or a region that includes at least about 100 to 200 amino acids, preferably 130 to 160, more preferably about 145 to 148 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "ubiquitin-conjugating enzyme domain," e.g., the ubiquitin-conjugating enzyme domain of human 27960 (e.g., residues 1-148 of SEQ ID NO: 73).

Detail Description Paragraph - DETX (4450):

[4268] To identify the presence of a "ubiquitin-conjugating enzyme" domain in a 27960 protein sequence, and to make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of HMMs (e.g., the Pfam database, release 2.1) using the default parameters (http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for MILPAT0063 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer et al. (1997) Proteins 28(3):405-420 and a detailed description of HMMs can be found, for example, in Gribskov et al. (1990) Meth. Enzymol. 183:146-159; Gribskov et al. (1987) Proc. Natl. Acad. Sci. USA 84:4355-4358; Krogh et al.(1994) J. Mol. Biol. 235:1501-1531; and Stultz et al.(1993) Protein Sci. 2:305-314, the contents of which are incorporated herein by reference. A search was performed against the HMM database resulting in the identification of a "ubiquitin-conjugating enzyme" domain in the amino acid sequence of human 27960 at about residues 1-148 of SEQ ID NO: 73.

Detail Description Paragraph - DETX (4500):

[4316] A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO: 72 or 74. For example, such a nucleic acid molecule can include a fragment that can be used as a probe or primer or a fragment encoding a portion of a 27960 protein, e.g., an immunogenic or biologically active portion of a 27960 protein. A fragment can comprise nucleotides 41 to 484 of SEQ ID NO: 72, which encodes a ubiquitin-conjugating enzyme domain of human 27960. The nucleotide sequence determined from the cloning of the 27960 gene allows for the generation of probes and primers designed for use in identifying and/or cloning other 27960 family members, or fragments thereof, as well as 27960 homologues, or fragments thereof, from other species.

Detail Description Paragraph - DETX (4539):

[4352] (vi) it has a ubiquitin-conjugating enzyme domain that has an overall sequence similarity of preferably about 70%, 80%, 90% or 95% with amino acid residues 1-148 of SEQ ID NO: 73; or

Detail Description Paragraph - DETX (4675):

[4476] Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene that encodes a ubiquitin-conjugating enzyme. Such disorders include, e.g., a disorder associated with the misexpression of 27960; a disorder of the neurological system, muscles, or immune system.

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ABSTRACT:

The present invention relates to the discovery in eukaryotic cells of a ubiquitin ligases. These proteins are referred to herein collectively as "pub" proteins for Protein UBiquitin ligase, and individually as h-pub1, h-pub2 and s-pub1 for the human pub1 and pub2 and Schizosaccharomyces pombe pub1 clones, respectively. Pub1 proteins apparently play a role in the ubiquitination of the mitotic activating tyrosine phosphatase cdc25, and thus they may regulate the progression of proliferation in eukaryotic cells by activating the cyclin dependent kinase complexes. In S. pombe, disruption of s-pub1 elevates the level of cdc25 protein in vivo increasing the activity of the tyrosine kinases, wee1 and mik1, required to arrest the cell-cycle. Loss of weel function in an S. pombe cell carrying a disruption in the s-pub1 gene results in a lethal premature entry into mitosis; such lethal phenotype can be rescued by the loss of cdc25 function. An ubiquitin thioester adduct of s-pub1 can be isolated from S. pombe and disruption of s-pub1 dramatically reduces ubiquitination of cdc25.

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Summary of Invention Paragraph - BSTX (78):

[0075] Polypeptides referred to herein as pub polypeptides preferably have an amino acid sequence corresponding to all or a portion of the pub1 amino acid sequence shown in SEQ ID No. 2 or in SEQ ID No.4, or the pub2 amino acid sequence shown in SEQ ID No. 6, or are homologous with one of these proteins, such as other human paralogs, or mammalian orthologs. In general, the biological activity of a pub polypeptide will be characterized as including the ability to transfer an ubiquitin molecule form the relevant ubiquitin conjugating enzyme (UBC) to a lysine residue of its target through a pub ubiquitin thioester intermediate; and an ability to translocate to specific phospholipid membranes in the presence of calcium. The above notwithstanding, the biological activity of a pub polypeptide may be characterized by one or more of the following attributes: an ability to regulate the cell-cycle of an eukaryotic cell, especially a mammalian cell (e.g., of a human cell), or a yeast cell such as a Schizosaccharomyces cell; an ability to modulate proliferation/cell growth of a eukaryotic cell; an ability to modulate entry of a mammalian or yeast cell into M phase; an ability to ubiquitinate a cell-cycle regulator, e.g. a mitotic activating tyrosine phosphatase, e.g. cdc25. Such activities may be manifested by the ability to control the steady state level of cdc25 phosphatase, and thus to control the degree of dephosphorylation of a cyclin dependent kinase. The pub polypeptides of the present invention may also function to modulate differentiation of cells/tissue. The subject polypeptides of this invention may also be capable of modulating cell growth or proliferation by influencing the action of other cellular proteins. A pub polypeptide can be a specific agonist of the function of the wild-type form of the protein, or can be a specific antagonist, such as a catalytically inactive mutant. Other biological activities of the subject pub proteins are described herein, or will be reasonably apparent to those skilled in the art in light of the present disclosure.

Summary of Invention Paragraph - BSTX (200):

[0197] The present invention also makes available S. pombe strains which contain a null pub mutation. As described herein, these strains can be complemented using human genes, and thus "humanized" yeast strains can be created for in vivo drug screen, e.g., which comprise a human pub homolog and (optionally) a human cdc25 phosphatase. The strain can be further manipulated to be "humanized" with respect to other biochemical steps in the pub1-mediated ubiquitination of the cdc25 fusion protein. For example, conditional inactivation of the relevant yeast UBC enzyme with concomitant expression of the human UBC homolog, or alternatively, replacement of other yeast genes involved in ubiquitination with their human homologs, provides a humanized system whereby the cdc25 protein can be ubiquitinated by a pub1-dependent mechanism which approximates the pub1-dependent ubiquitination that occurs in vertebrate cells.

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ABSTRACT:

The present invention describes a newly discovered ubiquitin conjugating enzyme homologue, called RATL1d6 herein, and its encoding polynucleotide, isolated and identified from activated T lymphocytes. Also described are expression vectors, host cells, agonists, antagonists, antisense molecules, and antibodies associated with the activity and use of the newly-discovered polynucleotide and/or polypeptide of the present invention. Methods for treating, diagnosing, preventing and screening for disorders related to the expression of the RATL1d6 ubiquitin conjugating enzyme polypeptide are described.

[0001] This application claims benefit to provisional application U.S. Serial No. 60/308,706, filed Jul. 30, 2001 and to provisional application U.S. Serial No. 60/244,688, filed Oct. 30, 2000.

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Title - TTL (1):
Polynucleotide encoding an activated human T-lymphocyte-derived protein related to ubiquitin conjugating enzyme

Summary of Invention Paragraph - BSTX (2):

[0002] The invention relates to the identification and isolation of a novel polynucleotide sequence and its encoded amino acid sequence defining a polypeptide expressed in activated human T-lymphocytes (T-cells) and having similarity to ubiquitin conjugating enzyme (UBC). The invention further relates to the use of the polynucleotides and the polypeptide in regulating cell growth and cell cycle progression, as well as in targeting the degradation of cellular proteins, and in the diagnosis, treatment and prevention of neoplastic diseases, immune disorders, and developmental and neuronal disorders and diseases.

Summary of Invention Paragraph - BSTX (8):

[0007] UBCs and families of genes encoding UBCs have been identified in a variety of eukaryotic genera, e.g., Saccharomyces, Dictyostelium, Drosophila, Caenorabditis elegans (C. elegans), Paramecia, as well as in mice and humans. UBCs or E2s are encoded by a large family of genes that are related to each other.

Summary of Invention Paragraph - BSTX (9):

[0008] The E2 ubiquitin-conjugating enzymes are important for substrate specificity in different UCS pathways. All E2 molecules have a conserved domain of approximately 16 kDa, called the UBC domain, that is at least 35% identical to all other E2s and contains a centrally located cysteine residue that is required for ubiquitin-enzyme thioester formation (S. Jentsch, supra). A highly conserved proline-rich element is located N-terminal to the active cysteine residue. Structural variations beyond this conserved domain are used to classify the E2 enzymes. The E2s of class I (E2-1) consist almost exclusively of the conserved UBC domain and include yeast E2-1 and UBCs 4, 5 and 7. These E2s are thought to require E3 to carry out their activities. (See, S. Jentsch, supra). UBC7 has been shown to recognize ubiquitin as a substrate and to form polyubiquitin chains in vitro (S. Van Nocker et al., 1996, J. Biol. Chem., 271:12150-12158). The E2s of class II (E2-2) have various unrelated C-terminal extensions that contribute to substrate specificity and cellular localization. The yeast E2-2 enzymes, UBC2 and UBC3, have highly acidic C-terminal extensions that promote interactions with basic substrates such as histones. Yeast UBC6 has a hydrophobic signal-anchor sequence that localizes the protein to the endoplasmic reticulum.

Summary of Invention Paragraph - BSTX (13):

[0011] The present invention provides a novel polynucleotide encoding a ubiquitin conjugating enzyme homologue, which was isolated from activated human T-cells, and hereinafter designated RATL1d6 ("Regulated in Activated T Lymphocytes 1d6"). RATL1d6 was discovered to be upregulated upon stimulation of Jurkat-line T cells and human peripheral blood T lymphocytes with antibodies directed against the CD3 and CD28 cell surface antigens. The RATL1d6 nucleic acid was identified in a subtraction library from activated human T lymphocytes as described herein. The RATL1d6 polypeptide encoded by the RATL1d6 nucleic acid sequence provided by this invention has similarity to ubiquitin conjugating enzyme.

Summary of Invention Paragraph - BSTX (14):

[0012] It is an object of the present invention to provide an isolated RATL1d6 polynucleotide as depicted in SEQ ID NO:1. In accordance with this invention, the isolated RATL1d6 polynucleotide encodes a ubiquitin conjugating enzyme comprising the amino acid sequence as set forth in SEQ ID NO:2. Fragments or portions of the RATL1d6 polynucleotide and polypeptide are also embraced by the invention. Preferably, the isolated RATL1 d6 polynucleotide or polypeptide, or fragment or portion thereof, is substantially purified.

Summary of Invention Paragraph - BSTX (17):

[0015] Yet another object of the present invention is to provide a ubiquitin conjugating enzyme polypeptide comprising an amino acid sequence having at least 80% to 90% sequence identity to the sequence set forth in SEQ ID NO:2.

Summary of Invention Paragraph - BSTX (18):
[0016] It is another object of the present invention to provide an isolated and substantially purified ubiquitin conjugating enzyme polypeptide encoded by the nucleic acid sequence of ATCC Deposit No. PTA-3745.

Summary of Invention Paragraph - BSTX (19):
[0017] It is another object of the present invention to provide a ubiquitin conjugating enzyme polypeptide whose amino acid sequence differs from SEQ ID NO:2 only by conservative substitutions.

Summary of Invention Paragraph - BSTX (22):
[0020] It is yet another object of the present invention to provide a ubiquitin conjugating enzyme polypeptide having at least 80% to 95% sequence identity to the sequence as set forth in SEQ ID NO:2.

Summary of Invention Paragraph - BSTX (23):
[0021] It is a further object of the present invention to provide a substantially purified ubiquitin conjugating enzyme fusion protein, wherein all or a portion of the RATL1d6 polypeptide is conjugated, coupled or linked to a heterologous polypeptide or peptide. More particularly, the invention provides an amino acid sequence having at least 80% to 95% sequence identity to the sequence as set forth in SEQ ID NO:2 and an amino acid sequence of an Fc portion of a human immunoglobulin protein. According to the present invention a ubiquitin conjugating enzyme fusion protein is provided in which the amino acid sequence differs from SEQ ID NO:2 only by conservative substitutions.

Summary of Invention Paragraph - BSTX (27):
[0025] It is also an object of the present invention to provide methods for producing a polypeptide comprising the amino acid sequence depicted in SEQ ID NO:2, or a fragment thereof, comprising: (a) cultivating a host cell containing an expression vector containing at least a functional fragment of the polynucleotide sequence encoding the RATL1d6 ubiquitin conjugating enzyme homologue according to this invention under conditions suitable for the expression of the polynucleotide; and (b) recovering the polypeptide from the host cell.

Brief Description of Drawings Paragraph - DRTX (2):
[0044] FIGS. 1A and 1B show separately the polynucleotide sequence of the RATL1d6 ubiquitin conjugating enzyme homologue (SEQ ID NO:1), (FIG. 1A), and the deduced amino acid sequence of the encoded RATL1d6 protein (FIG. 1B). The coding sequence (CDS) of RATL1d6 is 517 to 1782 of SEQ ID NO:1.

Brief Description of Drawings Paragraph - DRTX (4):
[0046] FIG. 3 shows the deduced amino acid sequence of the RATL1d6 ubiquitin conjugating enzyme polypeptide (SEQ ID NO:2). The predicted molecular weight of the RATL1d6 polypeptide encoded by the polynucleotide of SEQ ID NO:1 is MW=46.1 Kd.

Brief Description of Drawings Paragraph - DRTX (5):
[0047] FIG. 4 shows an alignment of the RATL1d6 ubiquitin conjugating enzyme (UBC) with hypothetical C. elegans and Drosophila orthologs F2H2.8 (Genbank Accession No: gi.vertline.3876332; SEQ ID NO:3) and EG:25E8.2 (Genbank

Accession No: gi.vertline.7290306; SEQ ID NO:4), respectively, and the E2 UBCs P52483/mouse UB6B (Genbank Accession No: gi.vertline.117850; SEQ ID NO:5); P27924/human UBC1/Huntingtin interacting protein (HIP), (Genbank Accession No: gi.vertline.14727922; SEQ ID NO:6); CAA72184/Drosophila UBCD4 (Genbank Accession No: gi.vertline.7294892; SEQ ID NO:7); and P14682/yeast UBC3/CDC34 (Genbank Accession No: gi.vertline.6320259; SEQ ID NO:8). The ubiquitin conjugating domain identified through a search of the PFAM database is boxed in RATL1d6. Residues that are identical in three or more of the aligned sequences are highlighted, and the conserved Cys residue which has been shown in UBCs to be involved in ubiquitin transfer through a thiol ester intermediate to target proteins is marked with a "". The % identity of RATL1d6 with C. elegans F2H2.8 is 42% over the entire peptide sequence; 47% with Drosophila EG:25E8.2 over the entire peptide sequence; and approximately 25% over the UBC domains only for the remaining proteins.

Detail Description Paragraph - DETX (2):

[0050] The present invention provides a novel isolated polynucleotide and encoded polypeptide, the expression of which is upregulated in human T lymphocytes that have been stimulated with anti-CD28 and anti-CD3 antibodies versus unstimulated T lymphocytes. This novel polypeptide is termed herein RATL1d6, an acronym for "Regulated in Activated T Lymphocytes 1d6", and is further characterized as a ubiquitin conjugating enzyme homologue.

Detail Description Paragraph - DETX (51):

[0098] In one of its embodiments, the present invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:2 as shown in FIG. 3. The RAT1d6 polypeptide is 422 amino acids in length and shares amino acid sequence homology to ubiquitin conjugating enzymes. The RATL1 d6 protein is thus characterized as a newly-discovered member of the UBC family isolated from activated T lymphocytes. In addition, FIG. 4 provides an alignment of the RATL1d6 polypeptide sequence with interspecies sequences comprising a ubiquitin conjugating enzyme (UBC) family of proteins having UBC domains.

Detail Description Paragraph - DETX (116):

[0162] In a related embodiment, an inhibitor of RATL1d6 function may be useful as an anti-cancer drug or agent with particular regard to the treatment of lymphoproliferative diseases, or as an immunosuppressive drug by functioning as a dominant negative to a UBC such as the RATL1d6 protein product, in a manner similar to the tumor susceptibility gene TSG101, which has been found to be mutated at a high frequency in human breast cancers. (C. P. Ponting et al., 1997, J. Mol. Med., 75:467-469; L. Li et al., 1997, Cell, 88:143-154). TSG101 has been implicated as a tumor suppressor gene, encoding a product having homology to ubiquitin conjugating enzymes, but lacking the conserved cysteine that is typically present in E2 proteins and is necessary for enzyme function. (C.P. Ponting et al., supra). TSG101 has also been reported to function as a dominant negative regulator of the ubiquitination of short-lived proteins (C. P. Ponting et al, supra and E. V. Koonin and R. A. Abagyan, 1997, Nature Genetics, 16:330-331). Accordingly, an antagonist of certain UBCs, such as RATL1d6 of the present invention, may also act in a manner similar to that of the TSG101 product and be utilized in the treatment of cancers, including T-cell and B-cell lymphoproliferative disorders and/or as an agent to suppress adverse immune system reactions.

Detail Description Paragraph - DETX (234):

[0277] To search the Drosophila orthologue of the human RATL1d6 gene, the RATL1d6 protein sequence was searched against the public Drosophila protein and genomic sequence database from GenBank, using the BLAST software (Altschul, S. F. et al., 1997, Nucleic Acids Res., 25:3389-3402). The Drosophila gene EG:25E8 (Genbank Accession No: AAF45767) was found to have the highest homology

with the human RATL1 d6 gene, with 48% identity at the amino acid level covering the majority of the gene. The Drosophila gene EG:25E8 was used to search against the public human protein and genomic sequence database from GenBank. Among all of the human genes, RATL1d6 was found to be most similar to the Drosophila EG:25E8 gene. The results of the database search indicate that EG:25E8 is the Drosophila orthologue of the human UBC enzyme RATL1d6 gene.

Detail Description Table CWU - DETL (7):

7TABLE 4 Sequence Listing Description SEQ ID NO: Description SEQ ID NO: 1 RATL1d6 nucleic acid sequence (FIG. 1) SEQ ID NO: 2 RATL1d6 polypeptide sequence (FIG. 3) SEQ ID NO: 3 C. elegans ortholog F2H2.8 (FIG. 4) SEQ ID NO: 4 Drosophila ortholog EG: 25E8.2 (FIG. 4) SEQ ID NO: 5 E2 UBC P52483/mouse UB6B (FIG. 4) SEQ ID NO: 6 P27924/human UBC1/Huntingtin interacting protein (HIP) (FIG. 4) SEQ ID NO: 7 CAA72184/Drosophila UBCD4 (FIG. 4) SEQ ID NO: 8 P14682/yeast UBC3/CDC34 (FIG. 4) SEQ ID NO: 9 PCR primer PY508, 5'-tgcagtgtctggctcggtgc-3' SEQ ID NO: 10 PCR primer PY509, 5'-ctgatctgcatgatcactgac-3' SEQ ID NO: 11 oligonucleotide PY495 5'-tccactgcaacatcacggagtcataccct- g- 3' SEQ ID NO: 12 oligonucleotide PY496 5'- atgcagtcgaactcgtgaatgacagtctgt-3' SEQ ID NO: 13 5' primer, N-terminal deletion (Example 12) SEQ ID NO: 14 3' primer, N-terminal deletion (Example 12) SEQ ID NO: 15 5' primer, C-terminal deletion (Example 12) SEQ ID NO: 16 3' primer, C-terminal deletion (Example 12) SEQ ID NO: 17 RATL1d6 polypeptide transmembrane domain SEQ ID NO: 18 PKC phosphorylation site polypeptide, GSVQATDRLMKEL SEQ ID NO: 19 PKC phosphorylation site polypeptide, IYRSQSFKGGNYA SEQ ID NO: 20 PKC phosphorylation site polypeptide, ILLNFSFKDNFPF SEQ ID NO: 21 PKC phosphorylation site polypeptide, TRAQQSYKSLVQI SEQ ID NO: 22 Casein kinase II phosphorylation site polypeptide, PAEQCTQEDVSSED SEQ ID NO: 23 Casein kinase II phosphorylation site polypeptide, TQEDVSSEDEDEEM SEQ ID NO: 24 Casein kinase II phosphorylation site polypeptide, QEDVSSEDEDEEMP SEQ ID NO: 25 Casein kinase II phosphorylation site polypeptide, AEGKKSEDDGIGKE SEQ ID NO: 26 Casein kinase II phosphorylation site polypeptide, ELVNDSLYDWNVKL SEQ ID NO: 27 Casein kinase II phosphorylation site polypeptide, ILLNFSFKDNFPFD SEQ ID NO: 28 Asparagine glycosylation site polypeptide, VRIHCNITESYPAV SEQ ID NO: 29 Asparagine glycosylation site polypeptide, AVELVNDSLYDWNV SEQ ID NO: 30 Asparagine glycosylation site polypeptide, DFILLNFSFKDNFP SEQ ID NO: 31 Asparagine glycosylation site polypeptide, VQFGANKSQYSLTR SEQ ID NO: 32 N-myristylation site polypeptide, QQPGPGQQLGGQGAAP SEQ ID NO: 33 N-myristylation site polypeptide, PGQQLGGQGAAPGAGG SEQ ID NO: 34 N-myristylation site polypeptide, QLGGQGAAPGAGGGPG SEQ ID NO: 35 N-myristylation site polypeptide, AAPGAGGGPGGGPGPG SEQ ID NO: 36 N-myristylation site polypeptide, APGAGGGPGGGPGPGP SEQ ID NO: 37 N-myristylation site polypeptide, EFLLAGAGGAGAGAAP SEQ ID NO: 38 N-myristylation site polypeptide, LLAGAGGAGAGAAPGP SEQ ID NO: 39 N-myristylation site polypeptide, LAGAGGAGAGAAPGPH SEQ ID NO: 40 N-myristylation site polypeptide, HLPPRGSVPGDPVRIH SEQ ID NO: 41 N-myristylation site polypeptide, QDYLNGAVSGSVQATD SEQ ID NO: 42 N-myristylation site polypeptide, NGAVSGSVQATDRLMK SEQ ID NO: 43 N-myristylation site polypeptide, SQSFKGGNYAVELVND SEQ ID NO: 44 N-myristylation site polypeptide, GYVLGGGAICMELLTK SEQ ID NO: 45 N-myristylation site polypeptide, ARVQFGANKSQYSLTR SEQ ID NO: 46 Amidation site polypeptide, EEEPAEGKKSEDDG SEQ ID NO: 47 UBC Domain of RATL1d6 SEQ ID NO: 48 RATL1d6 sense primer- expression profiling, 5'-aggatcatctccgacctgtg-3' (Example 13) SEQ ID NO: 49 RATL1d6 antisense primer- expression profiling, 5'-caagggttgatccagcatct-3' (Example 13) SEQ ID NO: 50 Promoter region of attacinD AMP forward primer (Example 14), 5'-atgaggcttggatcagcttt-3' SEQ ID NO: 51 Promoter region of attacinD AMP reversed primer (Example 14), 5'-cctgaagcctgacattccat-3' SEQ ID NO: 52 Forward primer-dsDNA, 5'-actgcagccgattcattaatg-3' (Example 14) SEQ ID NO:

53 Reverse primer-dsDNA, 5'- gaattaatacgactcactatagggagatat catacacatacgatttag-3' (Example 14) SEQ ID NO: 54 Forward primer-dsDNA, 5'-gaattaatacgactcactatagggagacat gattacgccaagctcgaa-3' (Example 14) SEQ ID NO: 55 Reverse primer-dsDNA, 5'-tgtaaaacgacggccagtgaa-3' (Example 14)

Claims Text - CLTX (2):

1. An isolated polynucleotide selected from the group consisting of: (a) an isolated polynucleotide encoding a ubiquitin conjugating enzyme homologue comprising the amino acid sequence as set forth in SEQ ID NO:2, or a fragment thereof; (b) an isolated polynucleotide comprising SEQ ID NO:1; (c) an isolated polynucleotide, or fragment thereof, encoding a ubiquitin conjugating enzyme amino acid sequence having at least 80% sequence identity with the sequence of SEQ ID NO:2; (d) an isolated polynucleotide having the nucleic acid sequence of ATCC Accession No. PTA-3745; (e) an isolated polynucleotide having the nucleic acid sequence according to nucleotides 517 to 1782 of SEQ ID NO:1, wherein said nucleotides encode a polypeptide of SEQ ID NO:2 minus the start codon; (f) an isolated polynucleotide having the nucleic acid sequence according to nucleotides 520 to 1782 of SEQ ID NO:1, wherein said nucleotides encode a polypeptide of SEQ ID NO:2 including the start codon; (g) an isolated polynucleotide which is fully complementary to the polynucleotide according to (a) through (f).

Claims Text - CLTX (7):

6. A substantially purified ubiquitin conjugating (UBC) enzyme polypeptide selected from the group consisting of: (a) a ubiquitin conjugating enzyme polypeptide having the amino acid sequence as set forth in SEQ ID NO:2; (b) a ubiquitin conjugating enzyme polypeptide comprising an amino acid sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO:2; (c) a polypeptide according to (a), wherein the amino acid sequence differs from SEQ ID NO:2 only by conservative substitutions; (d) a polypeptide according to (a), wherein the amino acid sequence has at least 90% sequence identity to the sequence set forth in SEQ ID NO:2; (e) an isolated ubiquitin conjugating enzyme polypeptide encoded by the nucleic acid sequence of ATCC Accession No. PTA-3745; (f) an isolated polypeptide having the amino acid sequence according to amino acids 2 to 422 of SEQ ID NO:2, wherein said amino acid encode a polypeptide of SEQ ID NO:2 minus the start methionine; (g) an isolated polypeptide having the amino acid sequence according to amino acids 1 to 422 of SEQ ID NO:2, wherein said amino acid encode a polypeptide of SEQ ID NO:2 including the start methionine; (h) an isolated polypeptide having the transmembrane domain region set forth in SEQ ID NO:17; and (i) a substantially purified fragment of the ubiquitin conjugating enzyme polypeptide according to any one of (a) to (h).

Claims Text - CLTX (8):

7. A substantially purified ubiquitin conjugating enzyme fusion protein comprising an amino acid sequence having at least 80% sequence identity to the sequence as set forth in SEQ ID NO:2 and an amino acid sequence of an Fc portion of a human immunoglobulin protein.

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TITLE: UBE2G2 ubiquitin-conjugating enzyme gene disruptions, compositions and methods related thereto

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INVENTOR-INFORMATION:

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ABSTRACT:

The present invention relates to transgenic animals, as well as compositions and methods relating to the characterization of gene function. Specifically, the present invention provides transgenic mice comprising mutations in a UBE2G gene. Such transgenic mice are useful as models for disease and for identifying agents that modulate gene expression and gene function, and as potential treatments for various disease states and disease conditions.

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 60/300,912, the entire contents of which are incorporated herein by reference.

---------- KWIC ---------

Title - TTL (1):
UBE2G2 ubiquitin-conjugating enzyme gene disruptions, compositions and methods related thereto

Summary of Invention Paragraph - BSTX (4):
[0003] Protein degradation is an essential mechanism for the maintenance of cellular homeostasis, in which excess or aberrant proteins are eliminated from the cell. In eukaryotes, conjugation of target proteins to ubiquitin is an essential step in the proteasome-dependent degradation process and is mediated by a family of ubiquitin conjugating enzymes (UBC). Several of these have been identified in a variety of organisms. Katsanis and Fisher (1998), Genomics 51: 128-131, stated that S. cerevisiae UBC7 is an endoplasmic reticulum-bound molecule whose active site faces the cytosol. UBC7 has been shown to confer resistance to cadmium and to participate in the degradation of specific yeast proteins. As part of an effort to generate a transcriptional map of human chromosome 21, Katsanis and Fisher (1998) identified UBE2G2 cDNAs. The

predicted 165-amino acid protein shares 60% sequence identity with yeast UBC7. The nucleotide sequence of UBE2G2 is 57% similar to that of UBE2G, another human UBC7 homolog. Northern blot analysis revealed that UBE2G2 is expressed ubiquitously as 2.9- and 7-kb mRNAs. The highest level of expression was seen in skeletal muscle.

Summary of Invention Paragraph - BSTX (6):
[0005] The complete 718 bp cds for the murine UBE2G2 (or E2G 2, or UBC7p homologue) gene has been deposited in GenBank (GenBank Accession No.: U93241; GI: 6649657).

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TITLE: Novel cyclin-selective ubiquitin carrier polypeptides

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US-CL-CURRENT: 435/226, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

Disclosed are novel human and clam ubiquitin carrier polypeptides involved in the ubiquitination of cyclins A and/or B. Also disclosed are inhibitors of such polypeptides, nucleic acids encoding such polypeptides and inhibitors, antibodies specific for such polypeptides, and methods of their use.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. Patent Application Ser. No. (HAZ-015), filed Mar. 18, 1997, which is related to Provisional Patent Application Ser. No. 60/014,492, filed Apr. 1, 1996, the disclosure of which is herein is incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (14):

[0013] As used herein, the term "involved in" means "which takes part in" and is meant to encompass the role played or function that a Ubc has during ubiquitination of cyclin A and/or B. This role includes an enzymatic activity required for transporting ubiquitin to cyclin A or B. The "Ubc-specific N-terminal extension" referred to in this aspect of the invention is used to describe a unique (outside of the conserved domain) amino acid sequence of at least 5, or preferably, at least 10, more preferably, at least 15, more preferably at least 20, more preferably, at least 25, most preferably between 30-32 amino acid residues having sequence homology to the unique amino acid sequence(s) found in clam E2-C, human UbcH10, and frog Ubc-x.

Summary of Invention Paragraph - BSTX (15):

[0014] In some embodiments, the Ubc is recombinantly produced. In other embodiments, fragments of the Ubc are provided which are enzymatically active and demonstrate the same or substantially similar ubiquitin carrier polypeptide function as the full length Ubc. As used herein a "fragment" of a molecule such as E2-C, UbcH10, or inhibitors thereof, refers to any smaller polypeptide subset of that molecule. In some embodiments, the Ubc is a clam or human Ubc. In some embodiments, the Ubc has an amino acid sequence with about 61-100%, more preferably, about 75-100%, and most preferably with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:1 or 3. By "homology" is meant sequence identity or similarity.

Summary of Invention Paragraph - BSTX (17):

[0016] In particular embodiments, the Ubc has the amino acid sequence set forth as SEQ ID NO:1 or 3. In yet other embodiments, the polypeptide is encoded by a nucleic acid hybridizable with a second nucleic acid set forth as SEQ ID NO:2 or 4. Preferably, the polypeptide is encoded by a nucleic acid hybridizable under stringent conditions with a second nucleic acid having SEQ ID NO:2 or 4. Stringent hybridization conditions are known by those with skill in the art (see, e.g., Ausebel et al., Protocols in Molecular Biology, John Wiley & Sons, Inc., New York, N.Y. (1989): hybridization in 50% formamide, high salt (either 5.times.SSC (20.times.: 3 M NaCl/0.3 M trisodium citrate) or 5.times.SSPE (20.times.: 3.6 M NaCl/0.2 M NaH.sub.2PO.sub.4/0.02 M EDTA, pH 7.7)), 5.times.Denhardt's solution, and 1% SDS) at low stringency: room temperature; moderate stringency: 42.degree. C.; and high stringency: 68.degree. C.

Summary of Invention Paragraph - BSTX (19):

[0018] In another aspect, the invention provides a nucleic acid encoding the Ubc's, and fragments thereof, of the invention as described above. In some embodiments, the nucleic acid is a cDNA, and in particular embodiments, the cDNA has the nucleotide sequence set forth as SEQ ID NO:2 or 4. In some embodiments, the nucleic acid of the invention encodes a human Ubc having an amino acid sequence with about 61-100% homology, preferably about 74-100%, and more preferably, with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:1. In other embodiments the nucleic acid of the invention encodes a clam Ubc having an amino acid sequence with about 61-100%, preferably with about 75-100%, and more preferably, with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:3. Also provided is a nucleic acid hybridizable under stringent conditions with a second nucleic acid having the nucleotide sequence set forth as SEQ ID NO:2 or 4.

Summary of Invention Paragraph - BSTX (20):

[0019] In another aspect, the present invention provides a selective inhibitor of Ubc polypeptide function. As used herein, the term "Ubc function" is meant to encompass the enzymatic transfer of ubiquitin from E1 to E2 and from E2 to a protein target, e.g., cyclin A or B. "Ubc function" also refers to the association of E2 and E3. The term "inhibitors of Ubc function" is meant

to include agents that block the transfer of ubiquitin from E1 to E2 and agents that block the transfer of ubiquitin from E2 to a protein target, e.g., cyclin A or B. As used herein, "inhibitors of Ubc function" is also meant to include agents that block association between E2 and E3. All such agents prevent cyclin ubiquitination. It is preferred that the agent be a selective inhibitor of Ubc function, more preferably wherein the Ubc is selected from the group consisting of clam E2-C, human UbcH10, and an enzymatically active fragment thereof. Suitable assays for measuring Ubc function according to the present invention include those which allow measurement of the formation of E-2-ubiquitin thiol ester, measurement of the formation of ubiquitin- or multi-ubiquitin-conjugates of a cyclin, or measurement of cyclin degradation. Assays that allow measurement of cell cycle progression may also be used according to the present invention.

Summary of Invention Paragraph - BSTX (24):

[0023] As used herein, a "selective inhibitor" is a compound which preferentially interferes with Ubc function. Preferably, the selective inhibitor reduces the enzymatic function of the novel Ubc's of the invention. In some embodiments, the inhibitor is a dominant negative mutant. As used herein, a "dominant negative mutant" is a polypeptide variant of a wild type Ubc with which it competes or interferes for its ubiquitin carrier function. Dominant negative mutants of the novel Ubc's of the invention inhibit cell cycle progression, blocking both the destruction of mitotic cyclins A and B, and the onset of anaphase. In some embodiments, the dominant negative mutant is recombinantly produced. In other embodiments, dominant negative mutants of the invention have a serine residue in place of a cysteine residue in a conserved region of the polypeptide. In specific embodiments, the dominant negative mutant of the invention comprises a serine residue at position 114 substituted for a cysteine residue. In some embodiments, the dominant negative mutant inhibits the function of a human or clam Ubc. The dominant negative mutant has an amino acid sequence with about 61-100%, preferably about 75-100%, and more preferably, about 94-100%, homology to the amino acid sequence set forth as SEQ ID NO:5 or 7 in some embodiments. In other embodiments, the dominant negative mutant is encoded by a nucleic acid hybridizable under stringent conditions with a second nucleic acid having the nucleotide sequence set forth as SEQ ID NO:6 or 8. In yet other embodiments, the invention provides a fragment of the dominant negative mutant which inhibits Ubc function.

Summary of Invention Paragraph - BSTX (26):

[0025] Kits useful for the ubiquitination and degradation of a cyclin are also provided by the invention. These kits include (a) a ubiquitin-human ubiquitin carrier polypeptide complex, wherein the ubiquitin carrier polypeptide is an isolated and purified, non-xenopal, Ubc involved in the ubiquitination of cyclin A and/or B, and having a Ubc-specific N-terminal extension. In preferred embodiments, the Ubc is clam E2-C, human UbcH10, or an enzymatically active fragment of clam E2-C or UbcH10; and (b) a ubiquitin ligase (E3).

Summary of Invention Paragraph - BSTX (27):

[0026] In some embodiments, the cyclin to be degraded is cyclin A or cyclin B and the ubiquitin-ubiquitin carrier polypeptide complex comprises human UbcH10 having an amino acid sequence set forth as SEQ ID NO:1. In another embodiment, the cyclin to be degraded is cyclin A or cyclin B and the ubiquitin-ubiquitin carrier polypeptide complex comprises clam E2-C having an amino acid sequence set forth as SEQ ID NO:3. In some embodiments, the ubiquitin-ubiquitin carrier protein complex comprises a Ubc having an amino acid sequence with about 61-100%, preferably about 75-100%, and more preferably, about 94-100% homology with the amino acid sequence set forth as

SEQ ID NO:1 or 3. In particular embodiments, the Ubc in the complex has the amino acid sequence set forth as SEQ ID NO:1 or 3. In yet other embodiments, the Ubc in the complex is encoded by a nucleic acid hybridizable under stringent conditions with a second nucleic acid set forth as SEQ ID NO:2 or 4. In some embodiments, the Ubc has an N-terminal extension which has about 61-100%, preferably about 75-100%, and more preferably about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:9 or 10. In particular embodiments, the Ubc in the complex has an N-terminal extension with an amino acid sequence set forth as SEQ ID NO:9 or 10.

Summary of Invention Paragraph - BSTX (29):

[0028] The invention also provides a method of ubiquitinating a cyclin and/or targeting a cyclin for degradation, comprising the step of contacting the cyclin with a ubiquitin-ubiquitin carrier protein complex, the ubiquitin carrier polypeptide being an isolated and purified non-xenopal Ubc involved in the ubiquitination of cyclin A and/or B, and having a Ubc-specific N-terminal extension; and a ubiquitin ligase (E3). In preferred embodiments, the Ubc is selected from the group consisting of clam E2-C, human UbcH10, and an enzymatically active fragment thereof. In some embodiments, the ubiquitin-ubiquitin carrier protein complex comprises a Ubc having an amino acid sequence with about 61-100%, preferably about 75-100%, and more preferably, with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:1 or 3. In particular embodiments, the Ubc in the complex has the amino acid sequence set forth as SEQ ID NO:1 or 3. In yet other embodiments, the Ubc in the complex is encoded by a nucleic acid hybridizable under stringent conditions with a second nucleic acid set forth as SEQ ID NO:2 or 4. In some embodiments, the Ubc has an N-terminal extension which has about 61-100% and more preferably, about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:9 or 10. In particular embodiments, the Ubc in the complex has an N-terminal extension with an amino acid sequence set forth as SEQ ID NO:9 or 10.

Summary of Invention Paragraph - BSTX (30):

[0029] A method of inhibiting Ubc function is also provided by the invention. In one embodiment, an inhibitor of a Ubc is administered to the cell in an amount sufficient to inhibit the Ubc function, e.g., by inhibiting the ubiquitination of a cyclin. In preferred embodiments, the inhibitor is a dominant negative mutant according to the invention and as described above. In some embodiments, the Ubc is a mutant clam E2-C. In other embodiments, the Ubc is a mutant human UbcH10. In some embodiments, the dominant negative mutant is recombinantly produced. In specific embodiments, the dominant negative mutant of the invention comprises a serine residue at position 114 substituted for a cysteine residue. In some embodiments, the dominant negative mutant inhibits the function of a human or clam Ubc. The dominant negative mutant has an amino acid sequence with about 61-100%, more preferably, about 75-100%, and most preferably, about 94-100%, homology to the amino acid sequence set forth as SEQ ID NO:5 or 7 in some embodiments. In other embodiments, the dominant negative mutant is encoded by a nucleic acid hybridizable under stringent conditions with a second nucleic acid having the nucleotide sequence set forth as SEQ ID NO:6 or 8. In yet other embodiments, the invention provides a fragment of the dominant negative mutant which inhibits Ubc function. In one preferred embodiment, the method of inhibiting Ubc function results in the inhibition of cell proliferation.

Summary of Invention Paragraph - BSTX (31):

[0030] The present invention further relates to a method of screening for compounds which inhibit Ubc function. In this method an assay is provided for measuring Ubc function, wherein the assay comprises a ubiquitin carrier polypeptide selected from the group consisting of a non-xenopal ubiquitin

carrier polypeptide involved in the ubiquitination of cyclin a and/or B and having a Ubc-specific N-terminal extension and an enzymatically active fragment thereof. The assay is performed in the presence and absence of a compound to-be-tested. The amount of change in Ubc function measured in the presence of the compound as compared to Ubc function measured in the absence of the compound is then determined, a reduction of Ubc function measured in the presence of the compound indicating that the compound is an inhibitor of Ubc function. In preferred embodiments, the ubiquitin carrier polypeptide is selected from the group consisting of clam E2-C, human UbcH10, and an enzymatically active fragment thereof. More preferably, the ubiquitin carrier polypeptide is isolated and purified.

Summary of Invention Paragraph - BSTX (33):
[0032] In preferred embodiments, the Ubc is selected from the group consisting of clam E2-C, human UbcH10, or an enzymatically active portion thereof. Preferably, the ubiquitin carrier polypeptide is isolated and purified. In some embodiments, the human UbcH10 or clam E2-C has an amino acid sequence with about 61-100%, preferably about 75-100%, and more preferably, with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:1 or 3, respectively. In particular embodiments, UbcH10 and E2-C have the amino acid sequences set forth as SEQ ID NO:1 and 3, respectively. In yet other embodiments, UbcH10 and E2-C are encoded by a nucleic acid hybridizable under stringent conditions, with a second nucleic acid set forth as SEQ ID NO:2 and 4, respectively. In some embodiments, UbcH10 has an N-terminal extension which has about 61-100%, preferably about 75-100%, and more preferably about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:9, and E2-C has an N-terminal extension which has about 61-100%, preferably about 75-100%, and more preferably, about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:10. In particular embodiments, the N-terminal extension of UbcH10 and E2-C has the amino acid sequence set forth as SEQ ID NO:9 and 10, respectively.

Brief Description of Drawings Paragraph - DRTX (23):
[0056] FIG. 13D is a representation of an autoradiogram demonstrating the effects of human and clam Ubc C(114)S mutants on the degradation of clam cyclin B;

Brief Description of Drawings Paragraph - DRTX (24):
[0057] FIG. 13E is a representation of an autoradiogram showing the reversal of the effects of human and clam Ubc C(114)S mutants (shown in FIG. 13D) by wild-type human Ubc, wherein the polypeptides were added at the concentrations indicated;

Detail Description Paragraph - DETX (10):
[0072] For cloning into a vector, suitable DNA preparations (either genomic or cDNA) are randomly sheared or enzymatically cleaved, respectively, and ligated into appropriate vectors to form a recombinant gene (either genomic or cDNA) library. A DNA sequence encoding Ubc may be inserted into a vector in accordance with conventional techniques, including blunt-ending or staggered-ending termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulation are disclosed by Sambrook et al., supra, and are well known in the art.

Detail Description Paragraph - DETX (13):
[0075] Oligonucleotide probes specific for Ubc which can be used to identify

clones to this protein can be designed from knowledge of the amino acid sequence of the corresponding Ubc. For example, the sequence of such oligonucleotide probes can be based upon the amino acid sequence of peptide fragment.

Detail Description Paragraph - DETX (14):
[0076] Using the genetic code, one or more different oligonucleotides can be identified, each of which would be capable of encoding Ubc polypeptides. The oligonucleotide, or set of oligonucleotides, containing a sequence most likely capable of identifying the Ubc gene sequence fragments is used to identify the sequence of a complementary set of oligonucleotides which is capable of hybridizing to the sequence, or set of sequences. An oligonucleotide sequence containing such a complementary sequence can be employed as a probe to identify and isolate Ubc gene sequence (for example, see Sambrook et al., supra).

Detail Description Paragraph - DETX (15):
[0077] The suitable oligonucleotide, or set of oligonucleotides, may be synthesized by means well known in the art (for example, see Sambrook et al., supra). Techniques of nucleic acid hybridization and clone identification are disclosed by Sambrook et al., supra. Those members of the above-described gene library which are found to be capable of such hybridization are then analyzed to determine the extent and nature of the Ubc encoding sequences which they contain.

Detail Description Paragraph - DETX (16):
[0078] In order to further characterize the Ubc-encoding DNA sequences, and in order to produce the recombinant protein, the DNA sequences are expressed. These sequences are capable of expressing a polypeptide if they contain expression control sequences "operably linked" to the nucleotide sequence which encodes the protein. The control sequences contain transcriptional regulatory information and such sequences.

Detail Description Paragraph - DETX (18):
[0080] The Ubc protein-encoding sequence and an operably linked promotor may be introduced into eucaryotic cells either as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Preferably, the introduced sequence is incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host.

Detail Description Paragraph - DETX (23):
[0085] The sequence obtained (SEQ ID NO:4) contains only one long open reading frame which initiates at the first methionine codon (FIG. 4). The size of the presumed translation product is 20 kD, in good agreement with the size of purified E2-C observed by SDS polyacrylamide gel electrophoresis. The encoded protein is clearly an E2, as demonstrated by its extensive alignment with other cloned Ubc's. Clam E2-C does not appear to be a Ubc2 homolog, since Ubc2's from several different species show much higher conserved sequence similarities within the family (.about.70%). The clam sequence contains a novel 30-32 amino acid N-terminal extension not found in any other Ubc besides the frog and human. Other unique regions include the adjacent sequence beginning at position 42 (TLLMSGD), and a short C-terminal extension (KYKTAQSDK). These features indicate that E2-C represents a novel Ubc.

Detail Description Paragraph - DETX (25):
[0087] The ability of the recombinant E2 to promote cyclin-ubiquitin ligation was tested in the presence of activated, partially purified E3-C/cyclosome complexes. As shown in FIGS. 9A and 9B, the recombinant E2 efficiently promoted this process, as compared to the action of natural E2-C.

The recombinant E2 stimulated cyclin ubiquitination at remarkable low concentrations: half-maximal activation was obtained with 0.05 .mu.M recombinant E2. Since it has been reported that Ubc4 can support cyclin B ubiquitination in a Xenopus egg extract (King et al. (1995) Cell 81:279-288) the activity of a recombinant human Ubc4 homolog, UbcH5 (Scheffner et al. (1994) Proc. Nat. Acad. Sci. USA 91:8797-8801) was also tested. As shown in FIGS. 9A and 9B (lane 4), UbcH5 caused some stimulation of cyclin-ubiquitin ligation by the clam E3-C/cyclosome complex, but the amount of conjugates formed and their size (which reflects the number of ubiquitin molecules attached to cyclin) were much lower than those obtained with the recombinant clam protein. Furthermore, in this experiment, the recombinant UbcH5 protein had to be added at a 20-fold higher molar concentration than the recombinant clam E2-C. Thus, at least in the clam oocyte system, Ubc4 supports cyclin ubiquitination much less efficiently than the new Ubc protein cloned here.

Detail Description Paragraph - DETX (26):

[0088] To examine the selectivity of the recombinant clam E2-C, the activity of these two E2's on the ligation of .sup.125I-ubiquitin to endogenous clam oocyte proteins was compared. Fraction 1A of clam oocytes contains a "non-specific" ubiquitin-protein ligase (E3) that can be separated from the cyclin-selective E3-C/cyclosome complex by its smaller size. This non-specific E3 ligates .sup.125I-ubiquitin to endogenous proteins in the presence of a mixture of clam E2's (Sudakin et al. (1995) Mol. Biol. Cell. 6:185-198). The protein substrates for ubiquitin ligation are presumably clam oocyte proteins present in the partially purified preparation of the non-specific E3. As shown in FIGS. 9A and 9B, UbcH5 strongly stimulated the ligation of .sup.125I-ubiquitin to high molecular weight conjugates in the presence of non-specific E3 from clam oocytes. This finding indicates that the human Ubc4 homolog can act with an appropriate clam E3. The formation of the high molecular weight conjugates required the addition of both UbcH5 and the non-specific E3. By contrast, the recombinant clam E2 had no significant influence on the formation of ubiquitin-protein conjugates by the non-specific E3 (FIG. 9B, lane 3). The only stable adduct formed in the presence of the recombinant clam E2-C is a 30 kD auto-ubiquitination product. The formation of this product does not require the presence of the non-specific E3. The amount of the product is higher in FIGS. 9A and 9B than in FIGS. 4A and 4B due to the longer incubation time. Its apparent 30 kD size in the denaturing conditions of gel electrophoresis is close to that expected for recombinant E2-ubiquitin adduct (29.5 kD). A similar auto-ubiquitination product with native E2-C is seen with a mix of natural E2-C and E2-A (FIGS. 9A and 9B, lane 2). In this case, some formation of high molecular weight ubiquitin-protein conjugates is seen. This is presumably due to the action of E2-A, which had-been found previously to coincide with a non-specific ubiquitination activity (Hershko et al. (1994). J. Biol. Chem. 269:4940-4946). Thus, by the criterion of the lack of its action with a non-specific E3, the recombinant clam E2-C is selective for the cyclin-ubiquitination system. Accordingly, the cDNA clone described here encodes the cyclin-selective E2-C that is responsible for the cell cycle stage-selective ubiquitination and destruction of the mitotic cyclins A and B.

Detail Description Paragraph - DETX (27):

[0089] In summary, these experiments provide the first identification, cloning, sequence, and in vitro analysis of an E2 (E2-C) that shows high selectivity for the mitotic cyclin B, a key regulator of the protein kinase Cdc2 which controls entry into and exit from mitosis (M phase) of the cell division cycle in all eucaryotes. In clam embryos, E2-C also functions in the ubiquitination of cyclin A In somatic cells of vertebrates (including humans) and other organisms, cyclin A is required for entry into both S phase (DNA synthesis) and M phase (mitosis). Comparisons of the E2-C sequence with those

of other Ubc's show that E2-C is a novel Ubc and reveals the presence of several unique sequence domains, including an N-terminal 32 amino acid extension not seen in any other Ubc family, a 7 amino acid region immediately downstream of this extension, and a short C-terminal extension. Clam E2-C has 65% sequence homology with the corresponding frog Ubc-x.

Detail Description Paragraph - DETX (31):
[0093] A human equivalent of clam E2-C, UbcH.sub.10, was also identified in a screen of a human HeLa cell cDNA library. This protein was cloned and sequenced as described in the Examples, below. The resulting cDNA sequence (SEQ ID NO:2) and corresponding protein sequence (SEQ ID NO:1) are shown in FIG. 5. This protein was identified as an E2-C homolog by alignment with the clam E2-C sequence. This Ubc has 80% sequence similarity with frog Ubc-x and 61% sequence homology with clam E2-C. UbcH10 and HsRad6A, the most closely related human Ubc family member, have 41% sequence homology. HsRad6A has an active sequence variant with 94% sequence homology with WT. Likewise, variants of clam and human Ubc's having from about 61-100%, preferably about 75-100%, and most preferably, about 94-100% sequence homology with their wild-type counterparts are expected to have ubiquitinating function.

Detail Description Paragraph - DETX (33):
[0095] Clam E2-C and human UbcH10 also share an N-terminal 32 amino acid extension which is also conserved in frog Ubc-x. The amino acid sequences of these N-terminal extensions derived from their respective cDNAs are set forth below in Table 3.

Detail Description Paragraph - DETX (44):
[0106] Mutational analyses of clam E2-C and human UbcH10 demonstrate that replacing various amino acids in the sequences with other amino acids may result in the formation of a Ubc that functions as a dominant negative inhibitor of wild type Ubc function.

Detail Description Paragraph - DETX (46):
[0108] FIGS. 16A and 16B show the cDNA and corresponding amino acid sequences of two dominant negative mutants in human and clam, respectively. In these mutants, changing the catalytic cysteine to serine at position 114 ("C(114)S") creates a Ubc that is an inhibitor of wild-type E2-C or UbcH10 function, as judged by the in vitro cyclin-ubiquitination assay described herein and shown in FIGS. 12A-12B. In this assay, .sup.125I-cyclin B was incubated with native E2-C and different concentrations of E2-C C(114)S mutant protein and E3C/cyclosome preparation and assayed for cyclin ubiquitination as described below in the Examples. The representative results shown in FIG. 13A demonstrate that wild-type UbcH10 catalyses cyclin ubiquitination in vitro, while UbcH10 C(114)S acts as a dominant negative in vitro (FIG. 13B).

Detail Description Paragraph - DETX (55):
[0117] Of course, since the amino acid sequence of other Ubc's are known (see, e.g., Wasugie et al. (1996) Nucleic Acids Res. 24:2005), dominant negative mutants of these Ubc's can be produced by replacing a cysteine residue in a conserved region of their amino acid sequence with a serine residue or even some other amino acid residue. For example, the cysteine residue at position 93 of Ubc9 can be replaced with a serine residue. Likewise, a cysteine residue in the conserved regions of any of Ubc4, Ubc5, Ubc6, Ubc7, or Ubc8 can be replaced with a serine residue to create a dominant negative mutant.

Detail Description Paragraph - DETX (56):
[0118] The availability of a dominant negative clam E2-C and human UbcH10 enable investigations into the function of E2-C and UbcH10 in the

ubiquitination of other proteins during the cell cycle or in other physiological processes. For example, such studies will determine if Ubc functions at just one cell cycle transition, namely exit from mitosis into G1 of the next cell cycle, or if it also functions at additional cell cycle transitions and, if so, which other proteins are ubiquitinated using this Ubc.

Detail Description Paragraph - DETX (200):
[0261] UbcH10 peptide compatible domains are identified as follows. The UbcH10 sequence is "mapped" onto the existing Ubc crystal structures (Cook et al. (1992) J. Biol. Chem. 267:15116-21; Cook et al. (1993) Biochem. 32:13809-13817) to identify regions on the surface. Peptides corresponding to these regions are then tested for their effect on cyclin-ubiquitination in vitro using the assay described above. Any peptides that block ubiquitination can be used as "lead" compounds for the rational design of therapeutic agents that are cell permeable and can potentially be used to block cyclin ubiquitination, and thus the cell cycle, in vivo.

Claims Text - CLTX (6):
6. The Ubc of claim 1 which is a human polypeptide

Claims Text - CLTX (7):
7. The Ubc of claim 6 having an amino acid sequence with about 61-100% homology to the amino acid sequence set forth as SEQ ID NO:1.

Claims Text - CLTX (8):
8. The Ubc of claim 7 having an amino acid sequence with at least 94-100% homology to the amino acid sequence set forth as SEQ ID NO:1.

Claims Text - CLTX (9):
9. The Ubc of claim 8 having the amino acid sequence set forth as SEQ ID NO:1.

Claims Text - CLTX (13):
13. The Ubc of claim 12 having an amino acid sequence with at least 61-100% homology to the amino acid sequence set forth as SEQ ID NO:3.

Claims Text - CLTX (14):
14. The Ubc of claim 13 having an amino acid sequence with at least 94-100% homology to the amino acid sequence set forth as SEQ ID NO:3.

Claims Text - CLTX (15):
15. The Ubc of claim 14 having the amino acid sequence set forth as SEQ ID NO:3.

Claims Text - CLTX (20):
20. The nucleic acid of claim 18 encoding a human Ubc having an amino acid sequence with about 61-100% homology with the amino acid sequence set forth as SEQ ID NO:1.

Claims Text - CLTX (21):
21. The nucleic acid of claim 20 encoding a human Ubc having an amino acid sequence with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:1.

Claims Text - CLTX (22):
22. The nucleic acid of claim 18 encoding a clam Ubc having an amino acid sequence with about 61-100% homology with the amino acid sequence set forth as SEQ ID NO:3.

Claims Text - CLTX (23):
23. The nucleic acid of claim 22 encoding a clam Ubc having an amino acid sequence with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:3.

Claims Text - CLTX (26):
26. The nucleic acid of claim 18 encoding a human Ubc having an amino acid sequence with about 61-100% homology with the amino acid sequence set forth as SEQ ID NO:1.

Claims Text - CLTX (27):
27. The nucleic acid of claim 26 encoding a human Ubc having an amino acid sequence with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:1.

Claims Text - CLTX (28):
28. The nucleic acid of claim 18 encoding a clam Ubc having an amino acid sequence with about 61-100% homology with the amino acid sequence set forth as SEQ ID NO:3.

Claims Text - CLTX (29):
29. The nucleic acid of claim 28 encoding a clam Ubc having an amino acid sequence with about 94-100% homology with the amino acid sequence set forth as SEQ ID NO:3.

Claims Text - CLTX (42):
42. The dominant negative mutant of claim 37 which inhibits the function of a human Ubc.

Claims Text - CLTX (56):
56. The kit of claim 55 wherein the Ubc in the complex has an amino acid sequence set forth as SEQ ID NO:1.

Claims Text - CLTX (58):
58. The kit of claim 57 wherein the Ubc in the complex has an amino acid sequence set forth as SEQ ID NO:3.

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TITLE: Three hybrid assay system

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

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APPL-NO: 10/ 091177

DATE FILED: March 4, 2002

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US-CL-CURRENT: 435/6, 435/7.1 , 530/350 , 536/23.1 , 536/5 , 552/570

ABSTRACT:

The invention provides compositions and methods for isolating ligand binding polypeptides for a user-specified ligand, and for isolating small molecule ligands for a user-specified target polypeptide using an improved class of hybrid ligand compounds.

REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional application No. 60/272,932, filed on Mar. 2, 2001; U.S. Provisional application No. 60/278,233, filed on Mar. 23, 2001; and U.S. Provisional application No. 60/329,437, filed on Oct. 15, 2001, the specifications of which are hereby incorporated by reference in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (75):

[0162] The "ubiquitins" are a class of proteins found in all eukaryotic cells. The ubiquitin polypeptide is characterized by a carboxy-terminal glycine residue that is activated by ATP to a high-energy thiol-ester intermediate in a reaction catalyzed by a ubiquitin-activating enzyme (E1). The activated ubiquitin is transferred to a substrate polypeptide via an isopeptide bond between the activated carboxy-terminus of ubiquitin and the epsilon-amino group of (a) lysine residue(s) in the protein substrate. This transfer requires the action of ubiquitin conjugating enzymes such as E2 and, in some instances, E3 activities. The ubiquitin modified substrate is thereby

altered in biological function, and, in some instances, becomes a substrate for components of the ubiquitin-dependent proteolytic machinery which includes both UBP enzymes as well as proteolytic proteins which are subunits of the proteasome. As used herein, the term "ubiquitin" includes within its scope all known as well as unidentified eukaryotic ubiquitin homologs of vertebrate or invertebrate origin which can be classified as equivalents of human ubiquitin. Examples of ubiquitin polypeptides as referred to herein include the human ubiquitin polypeptide which is encoded by the human ubiquitin encoding nucleic acid sequence (GenBank Accession Numbers: U49869, X04803). Equivalent ubiquitin polypeptide encoding nucleotide sequences are understood to include those sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants; as well as sequences which differ from the nucleotide sequence encoding the human ubiquitin coding sequence due to the degeneracy of the genetic code. Another example of a ubiquitin polypeptide as referred to herein is murine ubiquitin which is encoded by the murine ubiquitin encoding nucleic acid sequence (GenBank Accession Number: X51730). It will be readily apparent to the person skilled in the art how to modify the methods and reagents provided by the present invention to the use of ubiquitin polypeptides other than human ubiquitin.

Detail Description Paragraph - DETX (79):

[0166] The term "ubiquitin conjugation machinery" as used herein refers to a group of proteins which function in the ATP-dependent activation and transfer of ubiquitin to substrate proteins. The term thus encompasses: E1 enzymes, which transform the carboxy-terminal glycine of ubiquitin into a high energy thiol intermediate by an ATP-dependent reaction; E2 enzymes (the UBC genes), which transform the E1.about.S.about.Ubiquitin activated conjugate into an E2.about.S.about.Ubiquitin intermediate which acts as a ubiquitin donor to a substrate, another ubiquitin moiety (in a poly-ubiquitination reaction), or an E3; and the E3 enzymes (or ubiquitin ligases) which facilitate the transfer of an activated ubiquitin molecule from an E2 to a substrate molecule or to another ubiquitin moiety as part of a polyubiquitin chain. The term "ubiquitin conjugation machinery", as used herein, is further meant to include all known members of these groups as well as those members which have yet to be discovered or characterized but which are sufficiently related by homology to known ubiquitin conjugation enzymes so as to allow an individual skilled in the art to readily identify it as a member of this group. The term as used herein is meant to include novel ubiquitin activating enzymes which have yet to be discovered as well as those which function in the activation and conjugation of ubiquitin-like or ubiquitin-related polypeptides to their substrates and to poly-ubiquitin-like or poly-ubiquitin-related protein chains.

US-PAT-NO: 6734283

DOCUMENT-IDENTIFIER: US 6734283 B1

TITLE: Human proteins responsible for NEDD8 activation and conjugation

DATE-ISSUED: May 11, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chau; Vincent	Brookline	MA	N/A	N/A

APPL-NO: 09/ 216430

DATE FILED: December 18, 1998

PARENT-CASE:

This application is a continuation-in-part of provisional application Ser. No. 60/068,029, filed Dec. 19, 1997, and a continuation-in-part of provisional application Ser. No. 60/096,525, filed Aug. 12, 1998.

US-CL-CURRENT: 530/350, 435/6 , 435/7.1 , 536/23.1

ABSTRACT:

The invention relates to covalent modification of proteins through their conjugation with other proteins. More particularly, the invention relates to the modulation of such conjugation involving the protein NEDD8. The invention provides compositions and methods for detecting and/or modulating the activation and/or conjugation of NEDD8, as well as compositions and methods for discovering molecules which are useful in detecting and/or modulating the activation and/or conjugation of NEDD8. The present invention arises from the purification and characterization of novel NEDD8 activating and conjugating enzymes.

8 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Drawing Description Text - DRTX (8):
FIG. 7 shows the sequence alignment of NCE1 and NCE2 with known Ubc proteins.

Detailed Description Text - DETX (76):
The putative human homology of yeast Ubc12 was identified by searching the human EST database for clones having coding sequences that are homologous to the yeast protein. An initial search using the yeast protein sequence identified several clones. Clone AA261836, which contains a coding sequence very similar to a region of the yeast protein was used to search for further EST clones. The search led to the construction of a contiguous consensus

sequence from overlapping clones which predicts a gene to encode a protein having 183 amino acids, with a predicted molecular mass of 20899 Da. The contiguous nucleotide sequence was obtained using nested PCR on a human leukocyte cDNA library. The first PCR used primers having the sequences (SEQ ID NOS.: 29 and 30) GCAGGATGATCAAGCTGTTCTCGC (forward) and CGTGGCGGGGGTGGGTATGCGCCA (reversed). The second PCR used the primers (SEQ ID NOS.: 31 and 32) CGGGAATTCCATATGATCAAGCTGTTCTCGCTG (forward) and CGCCCAAGCTTCTATTTCAGGCAGCGCTCAAAG (reversed). The PCR product was digested with Nde1 and HindIII and ligated with similarly digested plasmid pT7-7. The resulting clone, pT7-7-UbcH12, was sequenced to determine the nucleotide sequence (SEQ ID NO 3) and deduced amino acid sequence (SEQ ID NO 4) shown in FIG. 1. FIG. 2 shows the alignment of NCE1 with yeast Ubc12. NCE1 shows 41% identity and 63% homology with yeast Ubc12.

Detailed Description Text - DETX (85):

The human EST database was searched using a query sequence (SEQ ID NO.: 34) HPNITETICLSLLREHSIDGTGWA. This is the sequence of clone AA306113 and bears similarity to the active site of proteins in the UBC protein family. Clones were identified which had sequences overlapping the sequence of clone AA306113. The identified sequences of the overlapping EST clones were aligned by the program CLUSTALW (See Thompson et al., Nucleic Acids Res. 22: 4673-4680 (1994), or by the program SeqMan (DNASTAR, Inc., Madison, Wis.) to yield a consensus sequence, CON1. CON 1 was used to perform searches for additional clones with overlapping sequences. The overlapping sequences yielded an open reading frame which encodes a protein of 185 amino acids (predicted molecular mass=21076 Da). Based upon homology to known human Ubc proteins, this gene is a member of the human Ubc gene family. The contiguous nucleotide sequence of NCE2 was obtained using nested PCR on a human leukocyte cDNA library. The first PCR used the primers (SEQ ID NOS.: 35 and 36) AGCCCAGGGTAAAGGCAGCA (forward) and CATGTTAGAGACAAACTGTA (reversed). The second PCR used the primers (SEQ ID NOS.: 37 and 38) GGGAATTCCATATGCTAACGCTAGCAAGTAA (forward) and CCATCGATTCATCTGGCATAACGTTTGA (reversed). The PCR product was then cloned into the NdeI/HindIII sites of pT7-7 to generate the plasmid pt7-7-HSUBC17. The sequence of the NCE2 gene and its deduced amino acid sequence are shown in FIG. 4. No close homolog exists in the yeast genome. The protein has 46% identity and 64% homology with a C. elegans gene (Genebank Accession #CE 275850) of unknown function (see FIG. 5).

Detailed Description Text - DETX (94):

The active site cysteine of a cloned NCE1 or NCE2 is assigned by examining the sequence alignment with known Ubc proteins (see FIG. 6 for alignment). The active site cysteine is replaced by a serine using standard site-specific mutagenesis. The mutant protein is expressed in bacteria and purified. The ability of the mutant protein to form a stable oxygen ester with NEDD8 is established as described in Examples 8 and 11 above, except that the bond formation is not liable in DTT. Dominant negative mutant activity is then established by introducing the mutant protein in increasing concentrations in an assay as described in Examples 8 and 11 above and demonstrating dose-dependent inhibition of NEDD8/NCE1 or NCE2 complex formation.

Detailed Description Paragraph Table - DETL (3):

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His Phe Pro Asp Pro Asn Lys Leu His Cys 50 55 60 ttt cag cta aca gta acc cca
gat gag ggt tac tac cag ggt gga aaa 240 Phe Gln Leu Thr Val Thr Pro Asp Glu
Gly Tyr Tyr Gln Gly Gly Lys 65 70 75 80 ttt cag ttt gaa act gaa gtt ccc gat
gcg tac aac atg gtg cct ccc 288 Phe Gln Phe Glu Thr Glu Val Pro Asp Ala Tyr
Asn Met Val Pro Pro 85 90 95 aaa gtg aaa tgc ctg acc aag atc tgg cac ccc aac
atc aca gag aca 336 Lys Val Lys Cys Leu Thr Lys Ile Trp His Pro Asn Ile Thr
Glu Thr 100 105 110 ggg gaa ata tgt ctg agt tta ttg aga gaa cat tca att gat
ggc act 384 Gly Glu Ile Cys Leu Ser Leu Leu Arg Glu His Ser Ile Asp Gly Thr
115 120 125 ggc tgg gct ccc aca aga aca tta aag gat gtc gtt tgg gga tta aac
432 Gly Trp Ala Pro Thr Arg Thr Leu Lys Asp Val Val Trp Gly Leu Asn 130 135
140 tct ttg ttt act gat ctt ttg aat ttt gat gat cca ctg aat att gaa 480 Ser
Leu Phe Thr Asp Leu Leu Asn Phe Asp Asp Pro Leu Asn Ile Glu 145 150 155 160
gct gca gaa cat cat ttg cgg gac aag gag gac ttc cgg aat aaa gtg 528 Ala Ala
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Leu 35 40 45 Pro Cys Thr Cys Lys Val His Phe Pro Asp Pro Asn Lys Leu His Cys
50 55 60 Phe Gln Leu Thr Val Thr Pro Asp Glu Gly Tyr Tyr Gln Gly Gly Lys 65
70 75 80 Phe Gln Phe Glu Thr Glu Val Pro Asp Ala Tyr Asn Met Val Pro Pro 85
90 95 Lys Val Lys Cys Leu Thr Lys Ile Trp His Pro Asn Ile Thr Glu Thr 100 105
110 Gly Glu Ile Cys Leu Ser Leu Leu Arg Glu His Ser Ile Asp Gly Thr 115 120
125 Gly Trp Ala Pro Thr Arg Thr Leu Lys Asp Val Val Trp Gly Leu Asn 130 135
140 Ser Leu Phe Thr Asp Leu Leu Asn Phe Asp Asp Pro Leu Asn Ile Glu 145 150
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Ser Ser Ser Ile Gln Pro Asn Leu Ser Ala Ala Arg Ile Arg Leu 20 25 30 Lys Arg
Asp Leu Asp Ser Leu Asp Leu Pro Pro Thr Val Thr Leu Asn 35 40 45 Val Ile Thr
Ser Pro Asp Ser Ala Asp Arg Ser Gln Ser Pro Lys Leu 50 55 60 Asn Val Cys Leu
Asn Ile Leu Arg Glu Asp Trp Ser Pro Ala Leu Asp 65 70 75 80 Leu Gln Ser Ile
Ile Thr Gly Leu Leu Phe Leu Phe Leu Glu Pro Asn 85 90 95 Pro Asn Asp Pro Leu
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Lys Leu Trp 35 40 45 His Leu Glu Val Pro Ser Thr Ser Cys Leu His Glu Leu Glu
Leu Thr 50 55 60 Val Thr Pro Gln Glu Gly Ile Tyr Arg Gly Gly Lys Phe Arg Phe
Lys 65 70 75 80 Ile Thr Val Pro Pro Glu Tyr Asn Asn Val Pro Pro Val Val Lys
Cys 85 90 95 Leu Thr Lys Val Trp His Pro Asn Ile Asn Glu Asp Gly Ser Ile Cys
100 105 110 Leu Ser Ile Leu Arg Gln Asn Ser Leu Asp Gln Tyr Gly Trp Arg Pro
115 120 125 Thr Arg Asn Leu Thr Asp Val Val His Gly Leu Val Ser Leu Phe Asn
130 135 140 Asp Leu Met Asp Phe Asn Asp Ala Leu Asn Ile Gln Ala Ala Gln Met
145 150 155 160 Trp Ser Trp Asn Arg Glu Ser Phe Asn His Arg Val Arg Glu Tyr
Ile 165 170 175 Ser Arg Tyr Cys 180 <200> SEQUENCE CHARACTERISTICS:
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Asp Leu Val 20 25 30 Asp Glu Asn Phe Thr Glu Leu Arg Gly Glu Ile Ala Gly Pro
Pro Asp 35 40 45 Thr Pro Tyr Glu Gly Gly Arg Tyr Gln Leu Glu Ile Lys Ile Pro
Glu 50 55 60 Thr Tyr Pro Phe Asn Pro Pro Lys Val Arg Phe Ile Thr Lys Ile Trp
65 70 75 80 His Pro Asn Ile Ser Ser Val Thr Gly Ala Ile Cys Leu Asp Leu Leu
85 90 95 Lys Asp Gln Trp Ala Ala Ala Met Thr Leu Arg Thr Val Leu Leu Ser 100
105 110 Leu Gln Ala Asp Leu Ala Ala Ala Glu Pro Asp Asp Pro Gln Asp Ala 115
120 125 Val Val Ala Asn Gln Tyr Lys Gln Asn Pro Glu Met Phe Lys Gln Thr 130
135 140 Ala Arg Leu Trp Ala His Val Tyr Ala Gly Ala Pro Val Ser Ser Pro 145
150 155 160 Glu Tyr Thr Lys Lys Ile Glu Asn Leu Cys Ala Met Gly Phe Asp Arg
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180 185 190 Ala Thr Glu Leu Leu Leu Ser Asn 195 200 <200> SEQUENCE
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Val Gly Val Ser Gly Ala Pro Ser Glu Asn Asn 20 25 30 Ile Met Gln Trp Asn Ala
Val Ile Phe Gly Pro Glu Gly Thr Pro Phe 35 40 45 Glu Asp Gly Thr Phe Lys Leu
Val Ile Glu Phe Ser Glu Glu Tyr Pro 50 55 60 Asn Lys Pro Pro Thr Val Arg Phe
Val Ser Lys Met Phe His Pro Asn 65 70 75 80 Val Tyr Ala Asp Gly Ser Ile Cys
Leu Asp Ile Leu Gln Asn Arg Trp 85 90 95 Ser Pro Thr Tyr Asp Val Ser Ser Ile
Leu Thr Ser Ile Gln Ser Asp 100 105 110 Leu Asp Glu Pro Asn Pro Asn Ser Pro
Ala Asn Ser Gln Ala Ala Gln 115 120 125 Leu Tyr Gln Glu Asn Lys Arg Glu Tyr
Glu Lys Arg Val Ser Ala Ile 130 135 140 Val Ile Gln Ser Trp Asn Asp Ser 145
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<220> FEATURE: <221> NAME/KEY: MISC_FEATURE <222> LOCATION:

(1)..(152) <223> OTHER INFORMATION: UBC2a <400> SEQUENCE: 13 Met Ser Thr Pro Ala Arg Arg Arg Leu Met Arg Asp Phe Lys Arg Leu

Detailed Description Paragraph Table - DETL (4):

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20 25 30 Ile Met Val Trp Asn Ala Val Ile Phe Gly Pro Glu Gly Thr Pro Phe 35
40 45 Gly Asp Gly Thr Phe Lys Leu Thr Ile Glu Phe Thr Glu Glu Tyr Pro 50 55
60 Asn Lys Pro Pro Thr Val Arg Phe Val Ser Lys Met Phe His Pro Asn 65 70 75
80 Val Tyr Ala Asp Gly Ser Ile Cys Leu Asp Ile Leu Gln Asn Arg Trp 85 90 95
Ser Pro Thr Tyr Asp Val Ser Ser Ile Leu Thr Ser Ile Gln Ser Asp 100 105 110
Leu Asp Glu Pro Asn Pro Asn Ser Pro Ala Asn Ser Gln Ala Ala Gln 115 120 125
Leu Tyr Gln Glu Asn Lys Arg Glu Tyr Glu Lys Arg Val Ser Ala Ile 130 135 140
Val Ile Gln Ser Trp Arg Asp Cys 145 150 <200> SEQUENCE CHARACTERISTICS:
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Arg Val Thr Leu 20 25 30 Val Asp Glu Gly Asp Leu Tyr Asn Trp Glu Val Ala Ile
Phe Gly Pro 35 40 45 Pro Asn Thr Tyr Tyr Glu Gly Gly Tyr Phe Lys Ala Arg Leu
Lys Phe 50 55 60 Pro Ile Asp Tyr Pro Tyr Ser Pro Pro Ala Phe Arg Phe Leu Thr
Lys 65 70 75 80 Met Trp His Pro Asn Ile Tyr Glu Thr Gly Asp Val Cys Ile Ser
Ile 85 90 95 Leu His Pro Pro Val Asp Asp Pro Gln Ser Gly Glu Leu Pro Ser Glu
100 105 110 Arg Trp Asn Pro Thr Gln Asn Val Arg Thr Ile Leu Leu Ser Val Ile
115 120 125 Ser Asp Leu Asn Glu Pro Asn Thr Phe Ser Pro Ala Asn Val Asp Ala
130 135 140 Ser Val Met Tyr Arg Lys Trp Lys Glu Ser Lys Gly Lys Asp Arg Glu
145 150 155 160 Tyr Thr Asp Ile Ile Arg Lys Gln Val Leu Gly Thr Lys Val Asp
Ala 165 170 175 Glu Arg Asp Gly Val Lys Val Pro Thr Thr Leu Ala Glu Tyr Cys
Val 180 185 190 Lys Thr Lys Ala Pro Ala Pro Asp Glu Gly Ser Asp Leu Phe Tyr
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Gly Pro Asn Asp Ser Pro Tyr Gln Gly Gly 35 40 45 Val Phe Phe Leu Thr Ile His
Phe Pro Thr Asp Tyr Pro Phe Lys Pro 50 55 60 Pro Lys Val Ala Phe Thr Thr Arg
Ile Tyr His Pro Asn Ile Asn Ser 65 70 75 80 Asn Gly Ser Ile Cys Leu Asp Ile
Leu Arg Ser Gln Trp Ser Pro Ala 85 90 95 Leu Thr Ile Ser Lys Val Leu Leu Ser
Ile Cys Ser Asp Leu Cys Asp 100 105 110 Pro Asn Pro Asp Asp Pro Leu Val Pro
Glu Ile Ala Arg Ile Tyr Lys 115 120 125 Thr Asp Arg Glu Lys Tyr Asn Arg Ile
Ala Arg Glu Trp Thr Gln Lys 130 135 140 Tyr Ala Met 145 <200>
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Gly Pro Asn Asp Ser Pro Tyr Gln Gly Gly 35 40 45 Val Phe Phe Leu Thr Ile His
Phe Pro Thr Asp Tyr Pro Phe Lys Pro 50 55 60 Pro Lys Val Ala Phe Thr Thr Arg
Ile Tyr His Pro Asn Ile Asn Ser 65 70 75 80 Asn Gly Ser Ile Cys Leu Asp Ile
Leu Arg Ser Gln Trp Ser Pro Ala 85 90 95 Leu Thr Ile Ser Lys Val Leu Leu Ser
Ile Cys Ser Asp Leu Cys Asp 100 105 110 Pro Asn Pro Asp Asp Pro Leu Val Pro
Glu Ile Ala Arg Ile Tyr Lys 115 120 125 Thr Asp Arg Asp Lys Tyr Asn Arg Ile
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Gly Pro Pro Asp Ser Ala Tyr Gln Gly Gly 35 40 45 Val Phe Phe Leu Thr Val His
Phe Pro Thr Asp Tyr Pro Phe Lys Pro 50 55 60 Pro Lys Ile Ala Phe Thr Thr Lys
Ile Tyr His Pro Asn Ile Asn Ser 65 70 75 80 Asn Gly Ser Ile Cys Leu Asp Ile
Leu Arg Ser Gln Trp Ser Pro Ala 85 90 95 Leu Thr Val Ser Lys Val Leu Leu Ser
Ile Cys Ser Asp Leu Thr Asp 100 105 110 Cys Asn Pro Asp Asp Pro Leu Val Pro
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Ser Lys Asn Ser Lys Leu Leu Ser Thr Ser 35 40 45 Ala Lys Arg Ile Gln Lys Glu
Leu Ala Asp Ile Thr Leu Asp Pro Pro 50 55 60 Pro Asn Cys Ser Ala Gly Pro Lys
Gly Asp Asn Ile Tyr Glu Trp Arg 65 70 75 80 Ser Thr Ile Leu Gly Pro Pro Gly
Ser Val Tyr Glu Gly Gly Val Phe 85 90 95 Phe Leu Asp Ile Thr Phe Thr Pro Glu
Tyr Pro Phe Lys Pro Pro Lys 100 105 110 Val Thr Phe Arg Thr Arg Ile Tyr His
Cys Asn Ile Asn Ser Gln Gly 115 120 125 Val Ile Cys Leu Asp Ile Leu Lys Asp
Asn Trp Ser Pro Ala Leu Thr 130 135 140 Ile Ser Lys Val Leu Leu Ser Ile Cys
Ser Asp Leu Thr Asp Cys Asn 145 150 155 160 Pro Ala Asp Pro Leu Val Gly Ser
Ile Ala Thr Gln Tyr Met Thr Asn 165 170 175 Arg Ala Glu His Asp Arg Met Ala
Arg Gln Trp Thr Lys Arg Tyr Ala 180 185 190 Thr <200> SEQUENCE
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Ile Val Pro Asp Asn Pro Pro Tyr Asp Lys 35 40 45 Gly Ala Phe Arg Ile Glu Ile
Asn Phe Pro Ala Glu Tyr Pro Phe Lys 50 55 60 Pro Pro Lys Ile Thr Phe Lys Thr
Lys Ile Tyr His Pro Asn Ile Asp 65 70 75 80 Glu Lys Gly Gln Val Cys Leu Pro
Val Ile Ser Ala Glu Asn Trp Lys 85 90 95 Pro Ala Thr Lys Thr Asp Gln Val Ile
Gln Ser Leu Ile Ala Asp Val 100 105 110 Asn Asp Pro Gln Pro Glu His Pro Leu
Arg Ala Asp Leu Ala Glu Glu 115 120 125 Tyr Ser Lys Asp Arg Lys Lys Phe Cys
Lys Asn Ala Glu Glu Phe Thr 130 135 140 Lys Lys Tyr Gly Glu Lys Arg Pro Val
Asp 145 150 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 20
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Ala Leu Leu Leu Pro Asp Gln Pro Pro Tyr His Leu Lys 35 40 45 Ala Phe Asn Leu
Arg Ile Ser Phe Pro Pro Glu Tyr Pro Phe Lys Pro 50 55 60 Pro Met Ile Lys Phe
Thr Thr Lys Ile Tyr His Pro Asn Val Asp Glu 65 70 75 80 Asn Gly Gln Ile Cys
Leu Pro Ile Ile Ser Ser Glu Asn Trp Lys Pro 85 90 95 Cys Thr Lys Thr Cys Gln
Val Leu Glu Ala Leu Asn Val Asp Val Asn 100 105 110 Arg Pro Asn Ile Arg Glu
Pro Leu Arg Met Asp Leu Ala Asp Leu Leu 115 120 125 Thr Gln Asn Pro Glu Leu
Phe Arg Lys Asn Ala Glu Glu Phe Thr Leu 130 135 140 Arg Phe Gly Val Asp Arg
Pro Ser 145 150 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO
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Asp Asp 20 25 30 Asn Asp Leu Tyr Arg Trp Glu Val Leu Ile Ile Gly Pro Pro Asp
Thr 35 40 45 Leu Tyr Glu Gly Gly Val Phe Lys Ala His Leu Thr Phe Pro Lys Asp
50 55 60 Tyr Pro Leu Arg Pro Pro Lys Met Lys Phe Ile Thr Glu Ile Trp His 65
70 75 80 Pro Asn Val Asp Lys Asn Gly Asp Val Cys Ile Ser Ile Leu His Glu 85
90 95 Pro Gly Glu Asp Lys Tyr Gly Tyr Glu Lys Pro Glu Glu Arg Trp Leu 100 105
110 Pro Ile His Thr Val Glu Thr Ile Met Ile Ser Val Ile Ser Met Leu 115 120
125 Ala Asp Pro Asn Gly Asp Ser Pro Ala Asn Val Asp Ala Ala Lys Glu 130 135
140 Trp Arg Glu Asp Arg Asn Gly Glu Phe Lys Arg Lys Val Ala Arg Cys 145 150
155 160 Val Arg Lys Ser Gln Glu Thr Ala Phe Glu 165 170 <200> SEQUENCE
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Ser Lys His Glu Val Thr Ile Leu Gly Gly Leu Asn 20 25 30 Glu Phe Val Val Lys
Phe Tyr Gly Pro Gln Gly Thr Pro Tyr Glu Gly 35 40 45 Gly Val Trp Lys Val Arg
Val Asp Leu Pro Asp Lys Tyr Pro Phe Lys 50 55 60 Ser Pro Ser Ile Gly Phe Met
Asn Lys Ile Phe His Pro Asn Ile Asp 65 70 75 80 Glu Ala Ser Gly Thr Val Cys
Leu Asp Val Ile Asn Gln Thr Trp Thr 85 90 95 Ala Leu Tyr Asp Leu Thr Asn Ile
Phe Glu Ser Phe Leu Pro Gln Leu 100 105 110 Leu Ala Tyr Pro Asn Pro Ile Asp
Pro Leu Asn Gly Asp Ala Ala Ala 115 120 125 Met Tyr Leu His Arg Pro Glu Glu
Tyr Lys Gln Lys Ile Lys Glu Tyr 130 135 140 Ile Gln Lys Tyr Ala Thr Glu Glu
Ala Leu Lys Glu Gln Glu Glu Gly 145 150 155 160 Thr Gly Asp Ser Ser Ser Glu
Ser Ser Met Ser Asp Phe Ser Glu Asp 165 170 175 Glu Ala Gln Asp Met Glu Leu
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Met Asn Leu Met Asn Trp Glu Cys Ala Ile Pro Gly Lys 35 40 45 Lys Gly Thr Pro
Trp Glu Gly Gly Leu Phe Lys Leu Arg Met Leu Phe 50 55 60 Lys Asp Asp Tyr Pro
Ser Ser Pro Pro Lys Cys Lys Phe Glu Pro Pro 65 70 75 80 Leu Phe His Pro Asn
Val Tyr Pro Ser Gly Thr Val Cys Leu Ser Ile 85 90 95 Leu Glu Glu Asp Lys Asp
Trp Arg Pro Ala Ile Thr Ile Lys Gln Ile 100 105 110 Leu Leu Gly Ile Gln Glu
Asp Leu Asn Glu Pro Asn Ile Gln Asp Pro 115 120 125 Ala Gln Ala Glu Ala Tyr
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Gln Glu Leu Met Thr Leu Met Met Ser Gly Asp 35 40 45 Lys Gly Ile Ser Ala Phe
Pro Glu Ser Asp Asn Leu Phe Lys Trp Val 50 55 60 Gly Thr Ile His Gly Ala Ala
Gly Thr Val Tyr Glu Asp Leu Arg Tyr 65 70 75 80 Lys Leu Ser Leu Glu Phe Pro
Ser Gly Tyr Pro Tyr Asn Ala Pro Thr 85 90 95 Val Lys Phe Leu Thr Pro Cys Tyr
His Pro Asn Val Asp Thr Gln Gly 100 105 110 Asn Ile Cys Leu Asp Ile Leu Lys
Glu Lys Trp Ser Ala Leu Tyr Asp 115 120 125 Val Arg Thr Ile Leu Leu Ser Ile
Gln Ser Asp Leu Gly Glu Pro Asn 130 135 140 Ile Asp Ser Pro Leu Asn Thr His
Ala Ala Glu Leu Trp Lys Asn Pro 145 150 155 160 Thr Ala Phe Lys Lys Tyr Leu
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FEATURE: <221> NAME/KEY: MISC_FEATURE <222> LOCATION: (1)..(152)
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US-PAT-NO: 6706867

DOCUMENT-IDENTIFIER: US 6706867 B1

TITLE: DNA array sequence selection

DATE-ISSUED: March 16, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lorenz; Matthias	Bethesda	MD	N/A	N/A

APPL-NO: 09/ 741238

DATE FILED: December 19, 2000

US-CL-CURRENT: 536/23.1, 435/6 , 536/24.3 , 536/24.31 , 536/24.32

ABSTRACT:

The present invention provides methods and compositions for the construction of custom cDNA microarrays. In particular, the methods involve the selection of relevant clusters based on knowledge and expression patterns using public database information and the identification of the best representative cDNA clones within the selected cluster. The methods facilitate the construction of custom microarrays suitable for use in any biotechnological art. In preferred embodiments, the present invention provides the the ImmunoChip.

8 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 29

---------- KWIC ---------

Detailed Description Paragraph Table - DETL (37):
regulated transcript (21 kDa) GENE Trt NM_009429 1120661 IC02400 UG75 Expression GENE Mm.14410 TITLE trypsin 2 GENE Try2 TRYP.vertline. NM_009430 516518 IC02401 UG75 Expression GENE Mm.6977 TITLE trypsin 4 GENE Try4 NM_011646 516518 IC02402 UG75 Expression GENE Mm.489 TITLE TPR-containing, SH2-binding phosphoprotein GENE Tsbp Tsp.vertline. NM_009431 603059 IC02403 UG75 Expression GENE Mm.30435 TITLE tuberous sclerosis 2 GENE Tsc2 tuberin.vertline. NM_011647 463053 IC02404 UG75 Expression GENE Mm.22688 TITLE tumor susceptibility gene 101 GENE tsg101 gi = 3184259 1889019 IC02405 UG75 Expression GENE Mm.27481 TITLE testis specific gene A12 GENE Tsga12 gi = 4589831 775253 IC02406 UG75 Expression GENE Mm.14644 TITLE translin GENE Tsn Eh domain, SH3 domain regulator of endocytosis 1.vertline.EH NM_011650 2182731 domain/SH3 domain-containing protein.vertline.EHSH1.vertline.Ese1.vertline.Sh3p17.vertline. IC02407 UG75 Expression GENE Mm.15312 TITLE thiosulfate sulfurtransferase, mitochondrial GENE Tst Rhodanese.vertline. NM_009437 905176 IC02408 UG75 Expression GENE Mm.22596 TITLE tissue specific transplantation antigen P35B GENE Tstap35b gi = 199585 1890218 IC02409 UG75 Expression GENE Mm.12194 TITLE tissue specific transplantation antigen P91A GENE Tstap91a AntP91a.vertline. gi = 191974 3155279 IC02410 UG75 Expression GENE Mm.3679 TITLE tetratricopeptide repeat

domain GENE Ttc3 TPRD.vertline. NM_009441 1920323 IC02411 UG76 LID366 GENE Mm.63510 TITLE transcription termination factor 1 GENE Ttf1 NM_009442 2749187 B cell IC02412 UG75 Expression GENE Mm.4142 TITLE trans-golgi network protein 1 GENE Ttgn1 TGN38.vertline.TGN38A.vertline. NM_009443 557037 IC02413 UG75 Expression GENE Mm.1904 TITLE Ttk protein kinase GENE Ttk esk.vertline.PYT.vertline. NM_009445 722497 IC02414 UG75 Expression GENE Mm.2108 TITLE transthyretin GENE Ttr NM_013697 1889331 IC02415 UG75 Expression GENE Mm.88110 TITLE tubulin alpha 1 GENE Tuba1 Tuba-1.vertline. NM_011653 1480912 IC02416 UG75 Expression GENE Mm.4591 TITLE tubulin alpha 2 GENE Tuba2 gi = 202209 1921237 IC02417 UG75 Expression GENE Mm.1155 TITLE tubulin alpha 4 GENE Tuba4 M[a]4.vertline. NM_009447 1431172 IC02418 UG75 Expression GENE Mm.22774 TITLE tubulin, beta 2 GENE Tubb2 M(beta)2.vertline. gi = 202226 3155071 IC02419 UG75 Expression GENE Mm.1703 TITLE tubulin, beta 5 GENE Tubb5 M(beta)5.vertline. NM_011655 1969782 IC02420 UG75 Expression GENE Mm.10214 TITLE tuftelin 1 GENE Tuft1 NM_011656 1066939 IC02421 UG75 Expression GENE Mm.12887 TITLE tubby-like protein 3 GENE Tulp3 NM_011657 2631246 IC02422 UG75 Expression GENE Mm.13885 TITLE tax-transcriptionally activated glycoprotein 1 GENE Txgp1 CD134.vertline.Ly-70.vertline.Ox-40 T-cell antigen.vertline.OX40.vertline. NM_011659 573690 IC02423 UG75 Expression GENE Mm.3264 TITLE TXK tyrosine kinase GENE Txk Rlk.vertline. NM_013698 621896 IC02424 UG75 Expression GENE Mm.90520 TITLE thioredoxin reductase 2 GENE Txnrd2 TrxR2.vertline. NM_013711 573010 IC02425 UG75 Expression GENE Mm.46301 TITLE TYRO protein tyrosine kinase binding protein GENE Tyrobp DAP12.vertline.KARAP.vertline.killer cell activating receptor associated NM_011662 553001 protein.vertline.Ly83.vertline. IC02426 UG75 Expression GENE Mm.14286 TITLE U2 small nuclear ribonucleoprotein auxiliary factor GENE U2af1-rs1 NM_011663 476501 (U2AF), 35 kDa, related sequence 1 IC02427 UG75 Expression GENE Mm.3358 TITLE U2 small nuclear ribonucleoprotein auxiliary factor GENE U2af2 gi = 55100 373587 (U2AF), 65 kDa IC02428 UG75 Expression GENE Mm.235 TITLE ubiquitin B GENE Ubb jaundice.vertline. NM_011664 1924032 IC02429 UG75 Expression GENE Mm.1485 TITLE ubiquitin-conjugating enzyme 4 GENE Ubce4 ubcM2.vertline. NM_009454 2599064 IC02430 UG75 Expression GENE Mm.4429 TITLE ubiquitin-conjugating enzyme 5 GENE Ubce5 ubcM2.vertline. NM_009455 616812 IC02431 UG75 Expression GENE Mm.3074 TITLE ubiquitin-conjugating enzyme 7 GENE Ubce7 UbcM4.vertline. NM_009456 2236301 IC02432 UG75 Expression GENE Mm.12892 TITLE ubiquitin-activating enzyme E1C GENE Ube1c UBA3.vertline.ubiquitin activating enzyme 3.vertline. NM_011666 1970743 IC02433 UG75 Expression GENE Mm.1104 TITLE ubiquitin-activating enzyme E1, Chr X GENE Ube1x A1S9.vertline.Sbx.vertline.Sxrb X-homolog.vertline.ts A1S9 defective DNA NM_009457 1480550 replication gene.vertline.Ube-1.vertline.ubiquitin 1.vertline. IC02434 UG75 Expression GENE Mm.1920 TITLE ubiquitin-conjugating enzyme E2B (RAD6 GENE Ube2b E2-14k.vertline.HR6B.vertline. NM_009458 1853271 homology) IC02435 UG75 Expression GENE Mm.5203 TITLE ubiquitin-conjugating enzyme E2H GENE Ube2h E2-20K.vertline. NM_009459 873865 IC02436 UG75 Expression GENE Mm.3268 TITLE ubiquitin-conjugating enzyme E2I GENE Ube2i Mmubc9.vertline.Ubce9.vertline. NM_011665 1080476 IC02437 UG75 Expression GENE Mm.9002 TITLE ubiquitin conjugating enzyme E3A GENE Ube3a E6-AP ubiquitin protein ligase.vertline.Hpve6a.vertline. NM_011668 1969606 IC02438 UG75 Expression GENE Mm.12953 TITLE ubiquitin hydrolyzing enzyme 1 GENE Ubh1 gi = 3213206 1958335 IC02439 UG75 Expression GENE Mm.7353 TITLE ubiquitin-like 1 GENE Ubl1 Pic1.vertline. NM_009460 1432536 IC02440 UG75 Expression GENE Mm.21846 TITLE ubiquitin-like 3 GENE Ubl3 HCG.vertline. NM_011908 2225589 IC02441 UG75 Expression GENE Mm.10731 TITLE ubiquitin-protein ligase e3 componen n-recognin GENE Ubr1 NM_009461 2286332 IC02442 UG75 Expression GENE Mm.100465 TITLE urocortin GENE Ucn gi = 193144 1051225 IC02443 UG75 Expression GENE Mm.12556 TITLE uncoupling protein 2, mitochondrial GENE Ucp2 NM_011671 1481400 IC02444 UG75 Expression GENE Mm.1830 TITLE ubiquitin fusion degradation 1 like GENE Ufd1l NM_011672 806772 IC02445 UG75 Expression GENE Mm.12971 TITLE

UDP-glucose ceramide glucosyltransferase GENE Ugcg GlcT-1.vertline. NM_011673 577841 IC02446 UG75 Expression GENE Mm.10709 TITLE UDP-glucose dehydrogenase GENE Ugdh Udpgdh.vertline. NM_009466 478525 IC02447 UG75 Expression GENE Mm.42472 TITLE UDP-glucuronosyltransferase 1 family, member 1 GENE Ugt1a1 glucuronosyltransferase, phenol-UDP.vertline.Gnt1.vertline.onosyltransferase, gi = 801898 2182188 phenol-UDP.vertline.UDP-glucuronosyltransferase 1a.vertline.Udpgt-1a.vertline. IC02448 UG75 Expression GENE Mm.29157 TITLE UDP-glucuronosyltransferase 2 family, member 5 GENE Ugt2b5 UDP-glucuronosyltransferase 3.vertline.Udpgt-3.vertline. NM_009467 1885728 IC02449 UG75 Expression GENE Mm.10826 TITLE uromodulin GENE Umod uromucoid, Tamm-Horsfall glycoprotein.vertline. gi = 927202 2236432 IC02450 UG75 Expression GENE Mm.2559 TITLE uridine monophosphate kinase GENE Umpk gi = 471980 875710 IC02451 UG75 Expression GENE Mm.802 TITLE uridine monophosphate synthetase GENE Umps gi = 200138 1223250 IC02452 UG75 Expression GENE Mm.27744 TITLE UNC-119 homolog (C. elegans) GENE Unc119h NM_011676 763372 IC02453 UG75 Expression GENE Mm.1393 TITLE uracil-DNA glycosylase GENE Ung UNG1.vertline.UNG2.vertline. NM_011677 406824 IC02454 UG75 Expression GENE Mm.3974 TITLE ubiquitous nuclear protein GENE Unp NM_011678 458851 IC02455 UG75 Expression GENE Mm.10865 TITLE urate oxidase GENE Uox NM_009474 1891137 IC02456 UG75 Expression GENE Mm.4610 TITLE uridine phosphorylase GENE Upp UdRPase.vertline.UPase.vertline. NM_009477 608590 IC02457 UG75 Expression GENE Mm.22494 TITLE uroporphyrinogen decarboxylase GENE Urod Neurod.vertline.neurogenic differentiation.vertline. NM_009478 669694 IC02458 UG75 Expression GENE Mm.3160 TITLE uroporphyrinogen III synthase GENE Uros3 NM_009479 2812163 IC02459 UG75 Expression GENE Mm.8 TITLE upstream transcription factor 1 GENE Usf1 upstream stimulatory factor.vertline. NM_009480 1180608 IC02460 UG75 Expression GENE Mm.15781 TITLE upstream transcription factor 2 GENE Usf2 NM_011680 537853 IC02461 UG75 Expression GENE Mm.27498 TITLE ubiquitin specific protease 18 GENE Usp18 UBP43.vertline. NM_011909 637880 IC02462 UG75 Expression GENE Mm.3571 TITLE ubiquitin specific protease 5 (isopeptidase T) GENE Usp5 ubiquitin carboxy terminal hydrolase T.vertline.Ucht.vertline. NM_013700 1247676 IC02463 UG75 Expression GENE Mm.396 TITLE ubiquitin specific protease 9, X chromosome GENE Usp9x Dffrx.vertline.Fafl.vertline.fat-facets like (Drosophila).vertline. NM_009481 1364128 IC02464 UG75 Expression GENE Mm.2258 TITLE uteroglobin GENE Utg Blastokinin.vertline.CC10.vertline.CCSP.vertline.clara cell secretory protein.vertline.UG.vertline. NM_011681 493435 IC02465 UG75 Expression GENE Mm.42222 TITLE utrophin GENE Utrn Dmdl.vertline.dystrophin-like.vertline.G-utrophin.vertline. gi = 1934962 1429197 IC02466 UG75 Expression GENE Mm.10218 TITLE ubiquitously transcribed tetratricopeptide repeat GENE Utx gi = 3021456 2158972 gene, X chromosome IC02467 UG75 Expression GENE Mm.28643 TITLE vesicle-associated membrane protein 2 GENE Vamp2 Syb-2.vertline.Syb2.vertline.synaptobrevin 2.vertline. NM_009497 2192348 IC02468 UG75 Expression GENE Mm.28420 TITLE valyl-tRNA synthetase 2 GENE Vars2 Bat-6.vertline.Bat6.vertline.D17H6S56E.vertline.DNA segment, Chr 17, human NM_011690 616783 D6S56E.vertline.G7a.vertline.HLA-B-associated transcript 6.vertline. IC02469 UG75 Expression GENE Mm.9684 TITLE vasodilator-stimulated phosphoprotein GENE Vasp gi = 1617401 586602 IC02470 UG75 Expression GENE Mm.5081 TITLE Vav2 oncogene GENE Vav2 NM_009500 875892 IC02471 UG75 Expression GENE Mm.8294 TITLE von Hippel-Lindau binding protein 1 GENE Vbp1 NM_011692 962690 IC02472 UG75 Expression GENE Mm.1021 TITLE vascular cell adhesion molecule 1 GENE Vcam1

Detailed Description Paragraph Table - DETL (44):

putative steroid dehydrogenase gi = 3142701 1749973 (KIK-I) mRNA, complete cds IC02869 00/02 Literature GENE Mm.7883 APC; Adenomatous Polyposis Coli protein NM_007462 1764323 IC02870 UG75 Expression GENE Mm.4485 TITLE Mus musculus RGL protein mRNA, complete cds gi = 537276 1853422 IC02871 UG75

Expression GENE Mm.4494 TITLE M.musculus mRNA for plakophilin 1 gi = 1707593 1885570 IC02872 UG75 Expression GENE Mm.14526 TITLE Mus musculus myosin light chain 2 mRNA, gi = 1675395 1885673 complete cds IC02873 00/02 Literature GENE Mm.1135 J05118 Mouse mast cell carboxypeptidase A mRNA, NM_007753 1885694 complete cds IC02874 UG75 Expression GENE Mm.30201 cds gi = 5931570 1885706 IC02875 UG75 Expression GENE Mm.6696 TITLE Mus musculus short-chain dehydrogenase CRAD2 gi = 3294554 1885707 mRNA, complete cds IC02876 UG75 Expression GENE Mm.1017 TITLE Mus musculus mRNA for sid478p, complete cds gi = 5931564 1885780 IC02877 UG75 Expression GENE Mm.37217 TITLE Mouse complement factor H-related protein gi = 192559 1886334 mRNA, complete cds, clone 13G1 IC02878 UG75 Expression GENE Mm.13694 PRECURSOR gi = 53458 1886580 IC02879 UG75 Expression GENE Mm.26782 TITLE Mus musculus zinc finger protein 94 (Zfp94) gi = 4097496 1886879 mRNA, complete cds IC02880 UG75 Expression GENE Mm.24565 TITLE Mus musculus Tim23 mRNA for translocase of gi = 4996327 1888596 inner mitochondrial membrane, complete cds IC02881 UG75 Expression GENE Mm.2379 TITLE M.musculus mRNA for ASM-like gi = 1552349 1888917 phosphodiesterase 3a IC02882 UG75 Expression GENE Mm.30076 TITLE Mus musculus nuclear RNA helicase Bat1 mRNA, gi = 4235115 1888985 complete cds IC02883 UG75 Expression GENE Mm.3485 TITLE Mus musculus hemopexin mRNA, partial cds gi = 1881767 1889460 IC02884 00/02 Literature GENE Mm.57204 Z46227 M.musculus gene for histone H1 gi = 559479 1889637 IC02885 UG75 Expression GENE Mm.29454 TITLE Mus musculus mRNA for epididymal secretory gi = 4038734 1890275 protein, complete cds IC02886 UG75 Expression GENE Mm.27230 TITLE Mus musculus mRNA for MSSP, complete cds gi = 4730905 1907931 IC02887 UG75 Expression GENE Mm.9052 TITLE Mus musculus putative membrane associated gi = 2801792 1908257 progesterone receptor component mRNA, complete cds IC02888 UG75 Expression GENE Mm.18729 TITLE Mus musculus beta prime coatomer protein gi = 2809536 1920325 mRNA, partial cds IC02889 UG75 Expression GENE Mm.3716 TITLE Mus musculus nucleolar protein (MSP58) mRNA, gi = 2384718 1921088 complete cds IC02890 UG75 Expression GENE Mm.44552 TITLE Mus musculus TXNRD1 mRNA for thioredoxin gi = 6467192 1921276 reductase 1, complete cds IC02891 UG75 Expression GENE Mm.27343 TITLE Mus musculus endomucin mRNA, complete cds gi = 4159992 1922158 IC02892 UG75 Expression GENE Mm.42795 TITLE Mus musculus mRNA for UBE-1c1, UBE-1c2, UBE- gi = 5668736 1922266 1c3, complete cds IC02893 UG75 Expression GENE Mm.16790 TITLE Mus musculus aldo-keto reductase mRNA, gi = 1698717 1922534 complete cds IC02894 UG75 Expression GENE Mm.29460 TITLE Mus musculus mRNA for adenylate kinase gi = 4760597 1923039 isozyme 2, complete cds IC02895 UG75 Expression GENE Mm.2485 TITLE M.musculus mRNA for C1D protein gi = 1185124 1924987 IC02896 UG75 Expression GENE Mm.2605 TITLE Mus musculus retinol binding protein (RBP) gi = 1515449 1924998 mRNA, complete cds IC02897 00/02 Literature GENE Mm.4563 MRE-binding transcription factor NM_008636 1969422 IC02898 UG75 Expression GENE Mm.29698 TITLE Mus musculus mRNA similar to human Sua1, gi = 5689241 1969668 complete cds IC02899 UG75 Expression GENE Mm.2982 TITLE Mus musculus hsp40 mRNA for heat shock gi = 6531981 1969809 protein 40, complete cds IC02900 UG75 Expression GENE Mm.37672 TITLE Mus musculus IKK-i mRNA for inducible IKappaB gi = 6012173 1969939 kinase, complete cds IC02901 UG75 Expression GENE Mm.10818 TITLE Mus musculus syntaxin 7 (Syn7) mRNA, complete gi = 3123923 1970284 cds IC02902 UG75 Expression GENE Mm.34562 TITLE Mus musculus mRNA for mSART-1(806), gi = 4126468 1970588 complete cds IC02903 UG75 Expression GENE Mm.28100 TITLE Mus musculus mRNA for L-specific multifunctional gi = 5830359 1970885 beta-oxidation protein, partial CDS IC02904 UG75 Expression GENE Mm.10133 TITLE Mus musculus putative ras effector Nore1 mRNA, gi = 2997697 1971377 complete cds IC02905 UG75 Expression GENE Mm.13430 TITLE Mus musculus unknown protein mRNA, complete gi = 2183322 1971615 cds IC02906 UG75 Expression GENE Mm.13162 TITLE Mus musculus ERG-associated protein ESET gi = 3644041 1971619 mRNA, complete cds IC02907 UG75 Expression GENE Mm.27783 TITLE Mus musculus mRNA for Ariadne protein, partial gi = 3925718 1972136 IC02908 00/02 Literature GENE Mm.14105 DNAse I NM_010061 1972180 IC02909 UG75

Expression GENE Mm.29546 TITLE Mus musculus BAF53a (Baf53a) mRNA, complete gi = 4001804 1972181 cds IC02910 UG75 Expression GENE Mm.331 TITLE Mouse mRNA for TI-225, complete cds gi = 3863211 1972252 IC02911 UG75 Expression GENE Mm.6856 TITLE Mus musculus pituitary tumor transforming gene gi = 3978251 2064867 protein (PTTG) mRNA, complete cds IC02912 UG75 Expression GENE Mm.30185 TITLE Mus musculus mRNA expressed in renal proximal gi = 5030943 2064927 tubles IC02913 UG75 Expression GENE Mm.30352 TITLE Mus musculus mRNA for choline/ethanolamine gi = 2897730 2064929 kinase, complete cds IC02914 UG75 Expression GENE Mm.1109 TITLE Mus musculus WW-domain binding protein 1 gi = 1777576 2064966 mRNA, complete cds IC02915 UG75 Expression GENE Mm.1570 TITLE Mus musculus ubiquitin conjugating enzyme gi = 1480741 2064976 (ubc4) mRNA, complete cds IC02916 UG75 Expression GENE Mm.18630 TITLE M.musculus mitochondrial mRNA for very-long- gi = 1279564 2076597 chain acyl-CoA dehydrogenase IC02917 00/02 Literature GENE Mm.57120 X16496 Murine H3.1 gene for histone H3.1 gi = 51326 2076773 IC02918 UG75 Expression GENE Mm.2112 TITLE Mus musculus peroxisomal/mitochondrial dienoyl- gi = 2606085 2076790 CoA isomerase ECH1p (Ech1) mRNA, complete cds IC02919 UG75 Expression GENE Mm.22676 TITLE Mouse replication-dependent histone H2A.1 gene gi = 1290268 2076817 IC02920 UG75 Expression GENE Mm.5567 TITLE Mus musculus carboxyl terminal LIM domain gi = 2996195 2076828 protein (Ldb3) mRNA, complete cds IC02921 UG75 Expression GENE Mm.3960 TITLE Mus musculus interferon regulatory factor 3 (mirf3) gi = 1658532 2088085 mRNA, alternatively spliced, complete cds IC02922 UG75 Expression GENE Mm.10681 TITLE Mus musculus osf-2 mRNA for osteoblast specific gi = 393321 2088188 factor 2, complete cds IC02923 UG75 Expression GENE Mm.25203 TITLE MEMBRANE-ASSOCIATED PROTEIN HEM-2 gi = 51135 2099309 IC02924 UG75 Expression GENE Mm.7013 TITLE Mus musculus KOI-4 gene, partial cds gi = 2623677 2099836 IC02925 UG75 Expression GENE Mm.38470 TITLE Mus musculus DNaseI precursor mRNA, complete gi = 437052 2123030 cds IC02926 UG75 Expression GENE Mm.5831 TITLE Mus musculus RNAse L inhibitor (Mu-RLI) mRNA, gi = 3273416 2123679 complete cds IC02927 UG75 Expression GENE Mm.3118 TITLE Mus musculus mRNA for actin-related protein 1 gi = 2804291 2135509 alpha-isoform, complete cds IC02928 UG75 Expression GENE Mm.28919 TITLE Mus musculus mRNA for sid23p, complete cds gi = 5931560 2135732 IC02929 UG75 Expression GENE Mm.29404 TITLE Mus musculus Ste-20 related kinase SPAK gi = 3851168 2135848 mRNA, complete cds IC02930 UG75 Expression GENE Mm.6670 TITLE Mus musculus AMP activated protein kinase gi = 2766684 2136735 mRNA, complete cds IC02931 UG75 Expression GENE Mm.28761 TITLE Mus musculus Ste20-like kinase mRNA, complete gi = 4101577 2158843 cds IC02932 UG75 Expression GENE Mm.34747 TITLE Mus musculus pancreas sodium bicarbonate gi = 3298571 2159587 cotransporter mRNA, complete cds IC02933 UG75 Expression GENE Mm.28510 TITLE Mus musculus mRNA

Detailed Description Paragraph Table - DETL (165):
similar to Ku70-binding protein gi = 2729153 719075 [H. sapiens] IC11402 UG75 Expression EST Mm.41432 TITLE ESTs, Moderately similar to calcium and DAG- gi = 2646138 1225837 regulated guanine nucleotide exchange factor I [M. musculus] IC11403 UG75 Expression EST Mm.41438 TITLE ESTs gi = 1715880 596607 IC11404 UG75 Expression EST Mm.41440 TITLE ESTs, Moderately similar to ZINC FINGER gi = 5474133 777635 PROTEIN MFG1 [Mus musculus] IC11405 UG75 Expression EST Mm.41441 TITLE ESTs gi = 4031923 1395475 IC11406 UG75 Expression EST Mm.41445 TITLE ESTs, Weakly similar to a thyroid hormone gi = 2074642 639681 responsive gene in human skin fibroblasts [H. sapiens] IC11407 UG75 Expression EST Mm.41447 HELICASE-DNA-BINDING PROTEIN CHD-1 gi = 4059293 472805 [Mus musculus] IC11408 UG75 Expression EST Mm.41449 TITLE ESTs gi = 4484613 637239 IC11409 UG75 Expression EST Mm.41450 TITLE ESTs, Weakly similar to F33G12.3 gene product gi = 6077329 2270207 [C. elegans] IC11410 UG75 Expression EST Mm.41451 TITLE ESTs gi = 2964833 1429736 IC11411 UG75 Expression EST Mm.41452 TITLE ESTs gi = 5265615 440673 IC11412 UG75 Expression EST Mm.41457 TITLE ESTs gi = 5491455 1229472 IC11413 UG75

Expression EST Mm.41458 TITLE ESTs gi = 2906930 617629 IC11414 UG75 Expression EST Mm.41460 TITLE ESTs, Weakly similar to proline-rich protein 15 gi = 1901309 573246 [R. norvegicus] IC11415 UG75 Expression EST Mm.41463 TITLE ESTs, Weakly similar to UbcM4 interacting gi = 1349119 622692 protein 28[M. musculus] IC11416 UG75 Expression EST Mm.41464 TITLE ESTs, Weakly similar to F53E2.1 [C. elegans] gi = 5478009 596333 IC11417 UG75 Expression EST Mm.41465 TITLE ESTs gi = 2918093 749066 IC11418 UG75 Expression EST Mm.41466 TITLE ESTs gi = 6084812 619794 IC11419 UG75 Expression EST Mm.41470 TITLE ESTs gi = 2720362 720988 IC11420 UG75 Expression EST Mm.41472 TITLE ESTs, Weakly similar to weakly similar to gi = 5910569 751084 S. cerevisiae CBP3 protein precursor [C. elegans] IC11421 UG75 Expression EST Mm.41477 TITLE ESTs gi = 5124644 1379152 IC11422 UG75 Expression EST Mm.41479 TITLE ESTs gi = 6084344 717922 IC11423 UG75 Expression EST Mm.41481 TITLE staufen (RNA-binding protein) homolog 2 GENE Stau2 gi = 5630091 974076 (Drosophila) IC11424 UG75 Expression EST Mm.41488 TITLE ESTs, Weakly similar to tumor suppressor gi = 6519322 720952 [H. sapiens] IC11425 UG75 Expression EST Mm.41489 TITLE ESTs gi = 5475739 636947 IC11426 UG75 Expression EST Mm.41491 TITLE ESTs gi = 4729741 750767 IC11427 UG75 Expression EST Mm.41492 TITLE ESTs gi = 5498115 388343 IC11428 UG75 Expression EST Mm.41493 TITLE ESTs, Weakly similar to SIG41 [M. musculus] gi = 4290263 577064 IC11429 UG75 Expression EST Mm.41495 TITLE ESTs, Moderately similar to NY-REN-45 antigen gi = 2306330 1295842 [H. sapiens] IC11430 UG75 Expression EST Mm.41496 TITLE ESTs, Weakly similar to OVCA2 gi = 2201624 637093 IC11431 UG75 Expression EST Mm.41497 TITLE ESTs gi = 4032483 1429526 IC11432 UG75 Expression EST Mm.41506 TITLE ESTs, Weakly similar to Dreg-2 protein gi = 5333251 1225415 [D. melanogaster] IC11433 UG75 Expression EST Mm.41507 TITLE ESTs gi = 4722609 622872 IC11434 UG75 Expression EST Mm.41508 TITLE ESTs, Weakly similar to Atu [D. melanogaster] gi = 5470877 1749177 IC11435 UG75 Expression EST Mm.41511 TITLE ESTs gi = 4726646 637305 IC11436 UG75 Expression EST Mm.41512 TITLE ESTs gi = 1826765 721091 IC11437 UG75 Expression EST Mm.41513 TITLE ESTs gi = 5477955 2649595 IC11438 UG75 Expression EST Mm.41518 TITLE ESTs gi = 1500816 973756 IC11439 UG75 Expression EST Mm.4152 TITLE ESTs gi = 2346360 1282649 IC11440 UG75 Expression EST Mm.41531 TITLE ESTs, Moderately similar to AAC-RICH MRNA gi = 1767936 551427 CLONE AAC3 PROTEIN [Dictyostelium discoideum] IC11441 UG75 Expression EST Mm.41532 TITLE ESTs gi = 1758938 622914 IC11442 UG75 Expression EST Mm.41533 TITLE ESTs gi = 1287904 1263816 IC11443 UG75 Expression EST Mm.41535 TITLE ESTs gi = 2893638 596707 IC11444 UG75 Expression EST Mm.41538 TITLE ESTs, Moderately similar to PLATELET BASIC gi = 1909080 752492 PROTEIN PRECURSOR [Sus scrofa] IC11445 UG75 Expression EST Mm.41539 TITLE ESTs, Moderately similar to HYPOTHETICAL 32.7 gi = 6084328 973543 KD PROTEIN IN NTH2-COQ1 INTERGENIC REGION [Saccharomyces cerevisiae] IC11446 UG75 Expression EST Mm.41540 TITLE ESTs gi = 2906762 623021 IC11447 UG75 Expression EST Mm.41541 TITLE ESTs, Moderately similar to protein DS 1,24K gi = 1682305 1379898 [H. sapiens] IC11448 UG75 Expression EST Mm.41543 TITLE ESTs, Weakly similar to C13F10.7 [C. elegans] gi = 2962269 1361464 IC11449 UG75 Expression EST Mm.41550 TITLE ESTs gi = 1407182 639462 IC11450 UG75 Expression EST Mm.41551 TITLE ESTs gi = 2625738 640283 IC11451 UG75 Expression EST Mm.41558 TITLE ESTs gi = 6078305 596956 IC11452 UG75 Expression EST Mm.41561 TITLE ESTs gi = 1768888 634438 IC11453 UG75 Expression EST Mm.41563 TITLE ESTs gi = 4293287 751687 IC11454 UG75 Expression EST Mm.41565 TITLE ESTs, Weakly similar to coded for by C. elegans gi = 5819448 598535 cDNA yk52e10.5 [C. elegans] IC11455 UG75 Expression EST Mm.41567 TITLE ESTs gi = 6938107 576453 IC11456 UG75 Expression EST Mm.41568 TITLE ESTs, Moderately similar to ZINC FINGER gi = 2813125 1327699 PROTEIN HF.12 [Homo sapiens] IC11457 UG75 Expression EST Mm.41578 TITLE ESTs gi = 6645880 1149773 IC11458 UG75 Expression EST Mm.41581 TITLE ESTs gi = 4031963 751132 IC11459 UG75 Expression EST Mm.41582 TITLE ESTs gi = 1919244 777434 IC11460 UG75 Expression EST Mm.41583 TITLE ESTs, Moderately similar to PTD015 [H. sapiens] gi = 2283198 1264501 IC11461 UG75 Expression EST Mm.41589 TITLE

ESTs gi = 1682380 577182 IC11462 UG75 Expression EST Mm.41593 TITLE ESTs, Weakly similar to latent TGF-beta binding gi = 1862632 481777 protein-2 [M. musculus] IC11463 UG75 Expression EST Mm.41596 TITLE ESTs gi = 1309508 750819 IC11464 UG75 Expression EST Mm.41603 TITLE ESTs gi = 3718680 636912 IC11465 UG75 Expression EST Mm.41608 TITLE ESTs gi = 3066891 1327559 IC11466 UG75 Expression EST Mm.41616 TITLE ESTs gi = 5488730 752204 IC11467 UG75 Expression EST Mm.41617 TITLE ESTs, Weakly similar to veli 3 [M. musculus] gi = 2592748 1148943 IC11468 UG75 Expression EST Mm.41618 TITLE ESTs gi = 5498334 1226067 IC11469 UG75 Expression EST Mm.41621 TITLE ESTs gi = 1379875 597916 IC11470 UG75 Expression EST Mm.41629 TITLE ESTs gi = 2560942 1750111 IC11471 UG75 Expression EST Mm.41631 TITLE ESTs gi = 2885752 1079562 IC11472 UG75 Expression EST Mm.41634 TITLE ESTs gi = 5597550 2655150 IC11473 UG75 Expression EST Mm.41635 TITLE ESTs, Moderately similar to KIAA0898 protein gi = 3682071 1293792 [H. sapiens] IC11474 UG75 Expression EST Mm.41636 TITLE ESTs

US-PAT-NO: 6667174

DOCUMENT-IDENTIFIER: US 6667174 B2

TITLE: Expression vectors containing hybrid ubiquitin promoters

DATE-ISSUED: December 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yew; Nelson	West Upton	MA	N/A	N/A

APPL-NO: 09/ 952152

DATE FILED: September 13, 2001

PARENT-CASE:

This Appln claims benefit of prov. No. 60/233,938 filed Sep. 18, 2000 and No. 60/259,567 filed Jan. 3, 2001.

US-CL-CURRENT: 435/320.1, 435/69.1 , 536/24.1

ABSTRACT:

Sustained transgene expression will be required for the vast majority of genetic diseases being considered for gene therapy. The initially high levels of expression attained with plasmid DNA (pDNA) vectors containing viral promoters, such as that from cytomegalovirus (CMV), decline precipitously to near background levels within 2 to 3 weeks. We have constructed pDNA vectors containing the human cellular ubiquitin B (Ub) promoter and evaluated their expression in the mouse lung. Cationic lipid-pDNA complexes were instilled intranasally (IN) or injected intravenously (IV) into immunodeficient BALB/c mice. Chloramphenicol acetyltransferase (CAT) reporter gene expression from the Ub promoter was initially very low at day 2 post-administration but by day 35 exceeded the level of expression attained from a CMV promoter vector by 4- to 9-fold. Appending a portion of the CMV enhancer 5' of the Ub promoter (CMV-Ub) increased CAT expression to nearly that of the CMV promoter and expression persisted in the lung for at least three months, with 50% of day 2 levels remaining at day 84. In the liver, expression from the CMV-Ub hybrid promoter was sustained for 42 days. Since previous studies have shown that eliminating immunostimulatory CpG motifs in pDNA vectors reduces their toxicity, we constructed a CpG deficient version of the CMV-Ub vector expressing alpha-galactosidase A, the enzyme that is deficient in Fabry disease, a lysosomal storage disorder. After IN or IV administration, levels of alpha-galactosidase A from this vector were not only undiminished but increased 500% to 1500% by day 35. These results suggest that CpG-reduced plasmid vectors containing a CMV-Ub hybrid promoter may provide the long-term expression and efficacy required for a practical gene therapeutic.

11 Claims, 8 Drawing figures

Exemplary Claim Number: 10

Number of Drawing Sheets: 8

---------- KWIC ---------

Brief Summary Text - BSTX (4):

Ubiquitin is an abundant, small, 76 amino acid protein that is expressed in all eukaryotic cells (Ciechanover et al. 2000; Wilkinson et al, 2000). The protein covalently attaches to abnormal, misfolded or short-lived proteins, marking them for destruction in proteasomes (Ciechanover, supra). Ubiquitin also associates with histones and may play a role in the regulation of gene expression (Spencer and Davie, 1999). The coding sequence is remarkably conserved evolutionarily, being identical from insect to man. There are at least three known ubiquitin genes in humans, named UbA, UbB, and UbC, which appear to contain one, three or nine precise direct repeats of the 76 amino acid coding unit, respectively (Baker and Board, Nucleic Acids Research, 15:443-463 (1987); Lund et al. 1985; Nenoi, et al 1996; and Wiborg et al., EMBO J., 4:755-759 (1985). The human UbB and UbC genes have been sequenced and shown to contain no introns within their coding regions, but each contain an intron in the 5' flanking region (Baker and Board, supra; Nenoi supra). The UbC promoter has been shown to provide high level, ubiquitous expression when inserted into transgenic mice and when incorporated into plasmid DNA vectors (Johansen et al., FEBS 267:289-294 (1990); Schorpp et al., Nucleic Acids Research, 24:1787-1788 (1996); Wulff et al., 1990).